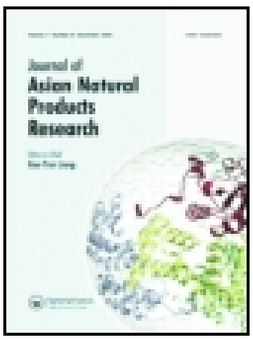

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Synthesis and antitumor activity evaluation of novel ursolic acid derivatives

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Eleven novel ursolic acid (**UA**) derivatives were designed and synthesized with modification at positions of C-2, C-3, and C-28 of **UA**. Their structures were confirmed by MS, ^{1}H NMR, and elemental analysis. Their *in vitro* cytotoxicities against various cancer cell lines (HeLa, HepG2, and BGC-823) were evaluated by MTT assay. The results indicated that all compounds could inhibit cell proliferation of HeLa, HepG2, and BGC-823 cells. Among them, compounds $\mathbf{I_3}$ and $\mathbf{I_4}$ showed more potent cytotoxicity on these three tumor cells than gefitinib (positive control), worthy to be studied further.

Keywords: ursolic acid derivatives; triterpenoid; synthesized; antitumor activity

1. Introduction

Ursolic acid (**UA**) is a pentacyclic triterpene compound isolated from leaves, berries, flowers, and fruits of many types of medicinal plants such as *Rosmarinus officinalis*, *Etiobotrya japonica,* and *Glecheoma hederaceae* [1,2]. It has been reported to possess a large range of pharmacological activities, including antitumor [3,4], anti-inflammatory [5,6], anti-HIV [7], anti-allergic, anti-bacterial, antiviral [8], and anti-angiogenic effects [9] in chick chorioallantotic membrane. In recent years, a large number of literature have reported that pentacyclic triterpenoids show a certain effect on tumor cells, and **UA** and their derivatives can inhibit tumor cell growth, induce apoptosis of tumor cells, and inhibit tumor angiogenesis [10–15]. These effects of **UA** are mediated through suppression of the expression of lipoxygenase, COX-2, MMP-9 [16], and iNOS, some of which are genes regulated by NF-KB [17], and the activities would be significantly enhanced by introduced methoxycarbonyl, carbonyl, or cyano-funtional groups. Based on the reports that the ester functionality at C-3 is essential for the pharmacological activities of pentacyclic triterpenes [18,19], a hydrogen donor group at either C-3 position and/or C-28 position of **UA** is essential for the cytotoxic activity [20]. Eleven **UA** derivatives with substitution at positions of C-2, C-3, and C-28 of **UA** have been synthesized and their cytotoxic activities have been evaluated *in vitro* against three cancer cell lines (HeLa, HepG2, and BGC-823). The results showed that compound $\mathbf{I_3}$ has more potent cytotoxicity on these three tumor cells than **UA**.

2. Results and discussion

2.1. *Chemical synthesis*

UA was used as the raw material, and eleven title compounds were designed and

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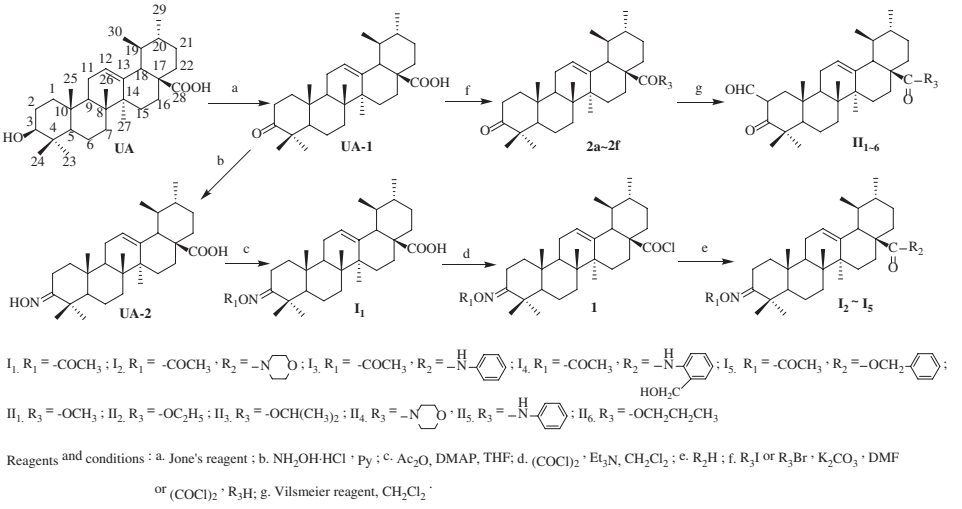

Scheme 1. Synthetic routes of target compounds.

synthesized by the structure modification at the positions C-2, C-3, and C-28 of **UA**. The synthetic routes of the title compounds are shown in Scheme 1. Compound **UA-1** was synthesized by the reaction of **UA** with Jone's reagent in acetone, then it was treated with NH_2·OH·HCl to give compound **UA-2**. **UA-2** was converted to compound $\mathbf{I_1}$ by the reaction with Ac_2O in the presence of 4-dimethylaminopyridine (DMAP) in tetrahydrofuran (THF). This intermediate was then condensed with the appropriate amino and phenol compounds in the presence of triethylamine to give the compounds $\mathbf{I_2}$–$\mathbf{I_5}$. The reaction of **UA-1** with bromoalkane in the presence of K_2CO_3 in *N*,*N*-dimethylformamide (DMF) gave carboxylic ester, then with Vilsmeier reagent gave compounds $\mathbf{II_1}$–$\mathbf{II_6}$. The title compounds were purified on a silica gel column with petroleum ether–ethyl acetate as eluents, and their structures were confirmed by MS, ^{1}H NMR, and elemental analysis, the results are summarized in Tables 1 and 2.

Table 1. MS and elemental analysis data of target compounds.

		Elemental analysis/found (calculated)			
Compound	ESI-MS (*m*/*z*)	C	H	N	O
$\mathbf{I_1}$	510.5[M − H]$^-$	74.92(75.11)	9.47(9.65)	2.68(2.74)	12.57(12.51)
$\mathbf{I_2}$	603.8[M + Na]$^+$	74.40(74.44)	9.75(9.72)	4.96(4.82)	11.31(11.02)
$\mathbf{I_3}$	587.1[M + H]$^+$	77.93(77.77)	9.41(9.28)	4.72(4.77)	8.25(8.18)
$\mathbf{I_4}$	617.4[M + H]$^+$	76.04(75.93)	9.28(9.15)	4.51(4.54)	10.11(10.37)
$\mathbf{I_5}$	602.3[M + H]$^+$	77.65(77.83)	9.17(9.21)	2.36(2.33)	10.67(10.63)
$\mathbf{II_1}$	497.6[M + H]$^+$	77.29(77.38)	9.66(9.74)	—	12.71(12.88)
$\mathbf{II_2}$	511.2[M + H]$^+$	77.49(77.60)	9.61(9.87)	—	12.64(12.53)
$\mathbf{II_3}$	525.9[M + H]$^+$	77.67(77.82)	9.83(9.99)	—	12.25(12.19)
$\mathbf{II_4}$	552.7[M + H]$^+$	76.22(76.18)	9.71(9.68)	2.46(2.54)	11.53(11.60)
$\mathbf{II_5}$	558.3[M + H]$^+$	79.71(79.67)	9.17(9.22)	2.28(2.51)	8.34(8.60)
$\mathbf{II_6}$	525.6[M + H]$^+$	77.59(77.82)	9.76(9.99)	—	12.03(12.19)

Table 2. ^{1}H NMR spectral data of target compounds.

Compound	^{1}H NMR ($CDCl_3$)
I_1	δ 11.0 (s, 1H, COOH), 5.35–5.29 (m, 1H, H-12), 2.26 (d, J = 8.6 Hz, 1H, H-18), 2.18 (s, 3H, CH_3COON), 1.38–1.34 (m, 1H, H-19), 1.32–1.29 (m, 1H, H-20), 1.27 (t, J = 7.5 Hz, 1H, H-5), 1.20 (t, J = 7.3 Hz, 1H, H-9), 1.15 (s, 3H, CH_3), 1.12 (s, 3H, CH_3), 1.05 (s, 3H, CH_3), 0.96 (s, 3H, CH_3), 0.89 (d, J = 7.0 Hz, 3H, CH_3), 0.85 (d, J = 7.1 Hz, 3H, CH_3), 0.79 (s, 3H, CH_3)
I_2	δ 5.33 (t, J = 3.5 Hz, 1H, H-12), 3.66 (t, J = 8.5 Hz, 2H, $NCH_2C\boldsymbol{H}_{2a}O$), 3.62 (t, J = 8.5 Hz, 2H, $NCH_2C\boldsymbol{H}_{2b}O$), 3.48 (t, J = 8.0 Hz, 2H, $NC\boldsymbol{H}_{2a}CH_2O$), 3.41 (t, J = 8.0 Hz, 2H, $NC\boldsymbol{H}_{2b}CH_2O$), 3.00 (d, J = 7.5 Hz, 1H, H-18), 2.15 (s, 3H, CH_3COON), 1.41–1.39 (m, 1H, H-19), 1.37–1.33 (m, 1H, H-20), 1.28 (t, J = 7.5 Hz, 1H, H-5), 1.22 (t, J = 7.0 Hz, 1H, H-9), 1.13 (s, 3H, CH_3), 1.09 (s, 3H, CH_3), 1.04 (s, 3H, CH_3), 0.93 (s, 3H, CH_3), 0.86 (d, J = 7.5 Hz, 3H, CH_3), 0.81 (d, J = 7.0 Hz, 3H, CH_3), 0.77 (s, 3H, CH_3)
I_3	δ 8.51 (s, 1H, NH), 7.53–7.21 (m, 5H, Ph), 5.31 (t, J = 4.0 Hz, 1H, H-12), 2.43 (d, J = 8.3 Hz, 1H, H-18), 2.32 (s, 3H, CH_3COON), 1.46–1.42 (m, 1H, H-19), 1.38–1.35 (m, 1H, H-20), 1.30 (t, J = 7.0 Hz, 1H, H-5), 1.23 (t, J = 7.1 Hz, 1H, H-9), 1.10 (s, 3H, CH_3), 1.06 (s, 3H, CH_3), 1.02 (s, 3H, CH_3), 0.95 (s, 3H, CH_3), 0.89 (d, J = 7.5 Hz, 3H, CH_3), 0.83 (d, J = 7.0 Hz, 3H, CH_3), 0.76 (s, 3H, CH_3)
I_4	δ 8.62 (s, 1H, NH), 7.68–7.26 (m, 4H, Ph), 5.34 (t, J = 3.6 Hz, 1H, H-12), 5.27 (s, 1H, OH), 4.64 (s, 2H, $C\boldsymbol{H}_2OH$), 2.50 (d, J = 7.6 Hz, 1H, H-18), 2.21 (s, 3H, CH_3COON), 1.43–1.40 (m, 1H, H-19), 1.38–1.35 (m, 1H, H-20), 1.26 (t, J = 7.5 Hz, 1H, H-5), 1.21 (t, J = 7.0 Hz, 1H, H-9), 1.08 (s, 3H, CH_3), 1.01 (s, 3H, CH_3), 0.95 (s, 3H, CH_3), 0.89 (s, 3H, CH_3), 0.85 (d, J = 8.1 Hz, 3H, CH_3), 0.80 (d, J = 7.5 Hz, 3H, CH_3), 0.75 (s, 3H, CH_3)
I_5	δ 7.52–7.21 (m, 5H, Ph), 5.29 (t, J = 3.6 Hz, 1H, H-12), 4.55 (s, 2H, CH_2O), 2.53 (d, J = 7.5 Hz, 1H, H-18), 2.20 (s, 3H, CH_3COON), 1.44–1.40 (m, 1H, H-19), 1.36–1.33 (m, 1H, H-20), 1.24 (t, J = 7.0 Hz, 1H, H-5), 1.18 (t, J = 6.5 Hz, 1H, H-9), 1.10 (s, 3H, CH_3), 1.05 (s, 3H, CH_3), 0.93 (s, 3H, CH_3), 0.87 (s, 3H, CH_3), 0.83 (d, J = 7.5 Hz, 3H, CH_3), 0.79 (d, J = 7.5 Hz, 3H, CH_3), 0.72 (s, 3H, CH_3)
II_1	δ 9.68 (s, 1H, CHO), 5.33 (t, J = 3.1 Hz, 1H, H-12), 3.76 (s, 3H, $COOCH_3$), 3.10 (t, J = 7.0 Hz, 1H, H-2), 2.52 (d, J = 6.0 Hz, 1H, H-18), 1.42–1.38 (m, 1H, H-19), 1.32–1.29 (m, 1H, H-20), 1.21 (t, J = 6.2 Hz, 1H, H-5), 1.15 (t, J = 7.8 Hz, 1H, H-9), 1.08 (s, 3H, CH_3), 1.03 (s, 3H, CH_3), 0.98 (s, 3H, CH_3), 0.95 (s, 3H, CH_3), 0.88 (d, J = 6.5 Hz, 3H, CH_3), 0.83 (d, J = 7.1 Hz, 3H, CH_3), 0.77 (s, 3H, CH_3)
II_2	δ 9.66 (s, 1H, CHO), 5.31 (t, J = 3.5 Hz, 1H, H-12), 4.08–4.05 (m, 2H, $COOC\boldsymbol{H}_2CH_3$), 3.12 (t, J = 7.0 Hz, 1H, H-2), 2.50 (d, J = 7.0 Hz, 1H, H-18), 1.48–1.44 (m, 1H, H-19), 1.35–1.32 (m, 1H, H-20), 1.25 (t, J = 8.7 Hz, 3H, $COOCH_2C\boldsymbol{H}_3$), 1.20 (t, J = 6.5 Hz, 1H, H-5), 1.13 (t, J = 7.0 Hz, 1H, H-9), 1.06 (s, 3H, CH_3), 1.01 (s, 3H, CH_3), 0.97 (s, 3H, CH_3), 0.93 (s, 3H, CH_3), 0.86 (d, J = 7.5 Hz, 3H, CH_3), 0.82 (d, J = 8.0 Hz, 3H, CH_3), 0.75 (s, 3H, CH_3)
II_3	δ 9.68 (s, 1H, CHO), 5.32 (t, J = 3.5 Hz, 1H, H-12), 4.24–4.21 (m, 1H, COOCH), 3.11 (t, J = 7.0 HZ, 1H, H-2), 2.52 (d, J = 6.5 Hz, 1H, H-18), 1.50–1.47 (m, 1H, H-19), 1.44–1.41 (m, 1H, H-20), 1.20 (d, J = 8.0 Hz, 3H, $COOCH(C\boldsymbol{H}_{3a})_2$), 1.17 (d, J = 8.0 Hz, 3H, $COOCH(C\boldsymbol{H}_{3b})_2$), 1.14 (t, J = 7.3 Hz, 1H, H-5), 1.10 (t, J = 7.0 Hz, 1H, H-9), 1.05 (s, 3H, CH_3), 1.01 (s, 3H, CH_3), 0.95 (s, 3H, CH_3), 0.91 (s, 3H, CH_3), 0.87 (d, J = 7.5 Hz, 3H, CH_3), 0.80 (d, J = 8.0 Hz, 3H, CH_3), 0.73 (s, 3H, CH_3)
II_4	δ 9.67 (s, 1H, CHO), 5.30 (t, J = 3.5 Hz, 1H, H-12), 3.65 (t, J = 8.5 Hz, 2H, $NCH_2C\boldsymbol{H}_{2a}O$), 3.62 (t, J = 8.5 Hz, 2H, $NCH_2C\boldsymbol{H}_{2b}O$), 3.50 (t, J = 8.0 Hz, 2H, $NC\boldsymbol{H}_{2a}CH_2O$), 3.46 (t, J = 8.0 Hz, 2H, $NC\boldsymbol{H}_{2b}CH_2O$), 3.10 (t, J = 7.0 HZ, 1H, H-2), 2.50 (d, J = 7.5 Hz, 1H, H-18), 1.51–1.48 (m, 1H, H-19), 1.46–1.42 (m, 1H, H-20), 1.19 (t, J = 8.0 Hz, 1H, H-5), 1.13 (t, J = 7.0 Hz, 1H, H-9), 1.09 (s, 3H, CH_3), 1.04 (s, 3H, CH_3), 0.93 (s, 3H, CH_3), 0.88 (s, 3H, CH_3), 0.85 (d, J = 7.0 Hz, 3H, CH_3), 0.81 (d, J = 8.0 Hz, 3H, CH_3), 0.74 (s, 3H, CH_3)

(*Continued*)

Table 2 – *continued*

Compound	^{1}H NMR ($CDCl_3$)
II$_5$	δ 9.68 (s, 1H, CHO), 8.49 (s, 1H, NH), 7.50–7.23 (m, 5H, Ph), 5.32 (t, J = 3.1 Hz, 1H, H-12), 3.15 (t, J = 7.0 Hz, 1H, H-2), 2.53 (d, J = 7.6 Hz, 1H, H-18), 1.49–1.45 (m, 1H, H-19), 1.41–1.38 (m, 1H, H-20), 1.21 (t, J = 8.0 Hz, 1H, H-5), 1.11 (t, J = 7.0 Hz, 1H, H-9), 1.08 (s, 3H, CH_3), 1.05 (s, 3H, CH_3), 0.99 (s, 3H, CH_3), 0.86 (s, 3H, CH_3), 0.83 (d, J = 7.5 Hz, 3H, CH_3), 0.79 (d, J = 8.5 Hz, 3H, CH_3), 0.76 (s, 3H, CH_3)
II$_6$	δ 9.66 (s, 1H, CHO), 5.30 (t, J = 3.8 Hz, 1H, H-12), 4.08 (t, 2H, J = 7.6 Hz, $COOCH_2CH_2CH_3$), 3.10 (t, J = 7.0 Hz, 1H, H-2), 2.51 (d, J = 7.5 Hz, 1H, H-18), 1.82–1.78 (m, 2H, $COOCH_2CH_2CH_3$), 1.43–1.40 (m, 1H, H-19), 1.38–1.34 (m, 1H, H-20), 1.22 (t, J = 6.5 Hz, 1H, H-5), 1.15 (t, J = 7.0 Hz, 1H, H-9), 1.07 (s, 3H, CH_3), 1.03 (s, 3H, CH_3), 0.98 (s, 3H, CH_3), 0.95 (t, J = 8.0 Hz, 3H, $COOCH_2CH_2CH_3$), 0.91 (s, 3H, CH_3), 0.86 (d, J = 7.5 Hz, 3H, CH_3), 0.82 (d, J = 8.0 Hz, 3H, CH_3), 0.77 (s, 3H, CH_3)

2.2. *Bioassays*

Antitumor activities of the title compounds were evaluated *in vitro* by the MTT method against HeLa, HepG2, and BGC-823 cell lines with **UA** and Gefitinib as the positive control. The results are summarized in Table 3.

As shown in Table 3, all the target compounds showed better inhibitory activity on HeLa, HepG2, and BGC-823 cell lines. Especially compounds **I$_3$** and **I$_4$** presented more significant inhibition to these kinds of tumor cells, and the inhibitory rates of compound **I$_3$** against HeLa, HepG2, and BGC-823 cell lines were 58.5%, 45.4%, 58.3%, while the inhibitory rates of compound **I$_4$** against these cells were 46.7%, 39.2%, 50.1%, respectively, significantly higher than that of **UA**, similar to the clinical medicine Gefitinib against HepG2 cells, significantly higher than Gefitinib against HeLa and BGC-823 cell lines. Modification of **UA** into acetyl oxyimino at C-3 position and amide group at C-28 position could improve the antitumor activity. The inhibitory activity of compound **II$_5$** on these kinds of tumor cells was higher than

Table 3. Inhibitory activity of the target compounds on the HeLa, HepG2, and BGC-823 cells proliferation.[a]

	Inhibition rate (%)			IC_{50} (μmol L^{-1})		
Compound	HeLa	HepG2	BGC-823	HeLa	HepG2	BGC-823
UA	12.3	11.8	10.2	>50	>50	>50
I$_1$	25.9	12.8	30.3	>50	>50	40.6
I$_2$	31.3	25.2	34.2	32.6	>50	35.2
I$_3$	58.5	45.4	58.3	9.25	21.2	8.06
I$_4$	46.7	39.2	50.1	13.8	23.7	9.15
I$_5$	28.1	26.6	36.5	>50	>50	32.9
II$_1$	16.2	24.2	20.1	>50	>50	>50
II$_2$	18.7	24.8	20.4	>50	>50	>50
II$_3$	21.5	20.7	25.2	>50	>50	>50
II$_4$	29.3	33.1	32.6	49.3	39.5	37.6
II$_5$	31.7	35.6	34.5	33.4	36.0	34.7
II$_6$	24.5	23.9	30.2	>50	>50	40.7
Gefitinib	42.6	46.2	46.0	17.1	20.7	19.3

[a] Inhibitory percentage of cells treated with each compound at a concentration of 10^{-5} mol l^{-1} for 72 h.

compound **UA**, so the introduced electron-withdrawing group (carbonyl group) at C-2 position of **UA** could also increase the antitumor activity. Thus, reasonable modification of **UA** into amide group at C-28 position increased the antitumor activity such as in compounds **I_3**, **I_4,** and **II_5**; alkyl side chains at C-3 such as alkanoyloxy imino chain, and electron-withdrawing group at C-2 position such as formyl group might be important to the inhibitory activity of tumor cell growth.

3. Conclusion

In this paper, eleven **UA** derivatives with the modification at C-2, C-3, and C-28 positions were prepared, and their antitumor activities on HeLa, HepG2, and BGC-823 cell lines were evaluated. As shown, our data suggested the transformation of the hydroxyl at C-3 and the carboxyl at C-28 of UA play a key role to improve the antitumor activity, and reasonable modifications at C-3 and C-28 of UA have a greater impact on the antitumor activity of UA. It will be the guiding significance for the next structure design and optimization, and lay a good foundation for the future research and development of high efficiency and low toxicity of anticancer drugs.

4. Experimental

4.1. Reagents and instruments

Melting points were determined in capillary tubes on a Büchi B-540 melting point apparatus produced by Broker Corporation (Flawil, Switzerland) and are uncorrected. NMR spectra were recorded on Bruker ARX-300 MHz spectrometers from Bruker Corporation (Ettlingen, Germany) and the solvent is $CDCl_3$, using trimethylsilane as an internal standard. ESI-MS were recorded on Thermo-Finnigan LCQ equipment from Thermo Finnigan (San Francisco, CA, USA). RPMI-1640 culture medium (containing 10% fetal bovine serum, 100 U ml^{-1} penicillin, 100 μg ml^{-1} streptomycin) was obtained from Shanghai Bioleaf Biotech Co., Ltd (Shanghai, China). Tetrazolium bromide salt (MTT), proteinase K (Proteinase K), and bovine serum albumin were obtained from Beyotime Institute of Biotechnology (Shanghai, China).

Tumor cells were cultivated on Sanyo MCO-175 carbon dioxide incubator from NAPCO Corporation (Gunmaken, Japan). SW-CJ-2FD Double Single surface clean bench was obtained from Suzhou Purification equipment Co., Ltd (Suzhou, China); Olympus IX70 inverted microscope from Olympus Corporation (Shinjuku, Tokyo, Japan); BIO-RAD MODEL680 microplate reader from North-West Medical Base Co. (St. Petersburg, Russia); TGL-16B low-speed desktop centrifuge from ShangHai Anting Scientific Instrument Factory (Shanghai, China). Thin-layer chromatography (TLC) was carried out with GF254, column chromatograph with silica gel (200–300 mesh) obtained from Qing-dao Marine Chemical Factory (Qingdao, China). The reagents were all of analytical grade or chemically pure.

4.2. Preparation of the compounds

*4.2.1. 3-Oxoursolic acid (**UA-1**)*

To a solution of **UA** (50 mg, 0.11 mmol) in acetone was titrated Jone's reagent (0.2 ml, freshly prepared) below 0°C, and the mixture was warmed to room temperature gradually, and stirred for 1 h. The end of reaction was monitored by TLC (eluent: petroleum ether–ethyl acetate; 3:1; the chromogenic agent was 10% sulfuric acid ethanol solution). Then, isopropanol (4 ml) was added to the mixture and stirred for 30 min at room temperature to quench the oxidation resistance. A small amount of saturated brine was added and then extracted with ethyl acetate for three times. The organic phases were combined and dried with

anhydrous sodium sulfate overnight, evaporated under reduced pressure to give a white solid powder **UA-1**, with a yield of 70.8%. m.p. 193.7–195.1°C; ESI-MS: *m/z* 455.2 $[M + H]^+$.

*4.2.2. 3-Oximeursolic acid (**UA-2**)*

UA-1 (50 mg, 0.11 mmol) was allowed to react with hydroxylamine hydrochloride (100 mg, 1.44 mmol) in pyridine, and the mixture was refluxed at 115°C for 1.5 h, then poured into ice water. A large amount of white precipitate was produced, filtered and the cake was washed with water, dried to give a white solid **UA-2**, with a yield of 82.96%. m.p. 215.6–218.1°C; ESI-MS: *m/z* 468.2 $[M - H]^-$.

*4.2.3. 3-Oxoursolic acid methyl ester (**2a**)*

UA-1 (100 mg, 0.22 mmol) was dissolved in DMF, then K_2CO_3 (30.0 mg, 0.22 mmol) and three drops of iodomethane were added at room temperature. The reaction end was monitored by TLC. After completion of reaction, the mixture was extracted with ethyl acetate and washed with saturated brine, dried over anhydrous magnesium sulfate. The solvent was evaporated under reduced pressure to give a white solid. The crude product was purified by column chromatography on silica gel with petroleum ether–ethyl acetate (10:1) to obtain compound **2a**, with a yield of 80.1%. m.p. 166.8–168.1°C; ESI-MS: *m/z* 469.5 $[M + H]^+$.

*4.2.4. 3-Oxoursolic acid ethyl ester (**2b**)*

According to the same method for compound **2a**, compound **2b** was prepared from **UA-1** (100 mg, 0.22 mmol), K_2CO_3 (30.0 mg, 0.22 mmol), and five drops of bromoethane in DMF. The crude product was purified by column chromatography on silica gel with petroleum ether–ethyl acetate (10:1) to obtain compound **2b**, with a yield of 76.3%. m.p. 153.2–164.7°C; ESI-MS: *m/z* 483.2 $[M + H]^+$.

*4.2.5. 3-Oxoursolic acid isopropyl ester (**2c**)*

According to the same method for compound **2a**, compound **2c** was prepared from **UA-1** (100 mg, 0.22 mmol) and K_2CO_3 (30.0 mg, 0.22 mmol), and five drops of isopropyl bromide in DMF. The crude product was purified by column chromatography on silica gel with petroleum ether–ethyl acetate (10:1), to obtain compound **2c**, with a yield of 66.8%. m.p. 143.6–145.2°C; ESI-MS: *m/z* 497.6 $[M + H]^+$.

*4.2.6. 3-Oxoursolic acid morpholine amide (**2d**)*

According to the same method for compound $\mathbf{I_2}$, compound **2d** was prepared from 3-oxoursolic acid chloride (100 mg, 0.21 mmol) and 5 ml of morpholine in DMF. The crude product was purified by column chromatography on silica gel with petroleum ether–ethyl acetate (6:1), to obtain compound **2d**, with a yield of 53.5%. m.p. 122.7–124.5°C; ESI-MS: *m/z* 524.7 $[M + H]^+$.

*4.2.7. 3-Oxoursolic acid aniline (**2e**)*

According to the same method for compound $\mathbf{I_2}$, compound **2e** was prepared from 3-oxoursolic acid chloride (100 mg, 0.21 mmol) and 6 ml of aniline in DMF. The crude product was purified by column chromatography on silica gel with petroleum ether–ethyl acetate (8:1) to obtain compound **2e**, with a yield of 61.2%. m.p. 128.3–129.5°C; ESI-MS: *m/z* 530.4 $[M + H]^+$.

*4.2.8. 3-Oxoursolic acid propyl ester (**2f**)*

According to the same method for compound **2a**, compound **2f** was prepared from **UA-1** (100 mg, 0.22 mmol) and

K_2CO_3 (30.0 mg, 0.22 mmol) and four drops of isopropyl bromide in DMF. The crude product was purified by column chromatography on silica gel with petroleum ether–ethyl acetate (10:1), to obtain compound **2f**, with a yield of 60.3%. m.p. 138.7–140.1°C; ESI-MS: *m/z* 497.3 $[M + H]^+$.

*4.2.9. 3-Acetoxyimineursolic acid (**I$_1$**)*

To a stirred solution of **UA-2** (50.0 mg, 0.11 mmol) in THF, pyridine (0.26 ml), acetic anhydride (0.340 g, 3.3 mmol), and a small amount of DMAP were added at room temperature, the reaction end was monitored by TLC (eluent:petroleum ether–ethyl acetate (3:1), and the chromogenic agent was 10% sulfuric acid ethanol solution). The resultant residue was partitioned in 3 ml water, then treated with 2 mol l^{-1} HCl to pH 3–4, and white precipitate was produced, filtered and the cake was washed with water to pH 7 and dried with anhydrous sodium sulfate. The crude was purified on a silica gel column chromatography (petroleum ether–ethyl acetate; 10:1) to give compound **I$_1$** as a white solid, with a yield of 73.8%, m. p. 89.5–91.4°C.

*4.2.10. 3-Acetoxyimineursolic acid morpholine amide (**I$_2$**)*

To a solution of compound **I$_1$** (50.0 mg, 0.099 mmol) in dichloromethane was added excess oxalyl chloride and the resultant mixture was stirred at room temperature for 20 h, the solvent and unreacted oxalyl chloride were removed under vacuum to give 3-acetoxyimineursolic acid chloride **1**. Compound **1** was dissolved completely in dichloromethane, then treated with triethylamine to pH 9–10, stirred for 5 min, added morpholine (20.8 mg, 0.15 mmol), and reacted at room temperature. The reaction end was monitored by TLC. The resultant residue was partitioned in 2 ml water, then treated with 2 mol l^{-1} HCl to pH 3–4, CH_2Cl_2 was removed under vacuum to precipitate white solid. After filtration, the cake was washed with water to pH 7 and dried with anhydrous sodium sulfate. The crude was purified on a silica gel column with petroleum ether–ethyl acetate (15:1) to give a white powder **I$_2$**, with a yield of 36.0%. m.p. 129.8–131.2°C.

*4.2.11. 3-Acetoxyimineursolic acid aniline amide (**I$_3$**)*

According to the same method for compound **I$_2$**, compound **I$_3$** was prepared from **I$_1$** (50.0 mg, 0.099 mmol) and aniline (20.0 mg, 0.21 mmol) through the corresponding acyl chloride (**1**). The solid was purified by column chromatography (petroleum ether–ethyl acetate; 10:1) to give compound **I$_3$** as a white powder, with a yield of 36.5%. m.p. 94.4–96.7°C.

*4.2.12. 3-Acetoxyimineursolic acid-2-hydroxymethyl aniline (**I$_4$**)*

According to the same method for compound **I$_2$**, compound **I$_4$** was prepared from **I$_1$** (50.0 mg, 0.099 mmol) and 2-aminophenyl-methanol (30.5 mg, 0.24 mmol) through the corresponding acyl chloride (**1**). The solid was purified by column chromatography (petroleum ether–ethyl acetate; 10:1) to give compound **I$_4$** as a white solid, with a yield of 38.9%. m.p. 78.4–80.6°C.

*4.2.13. 3-Acetoxyimineursolic acid benzyl ester (**I$_5$**)*

According to the same method for compound **I$_2$**, compound **I$_5$** was prepared from **I$_1$** (50.0 mg, 0.099 mmol) and benzyl alcohol (25.6 mg, 0.24 mmol) through the corresponding acyl chloride (**1**). The solid was purified by column chromatography (petroleum ether–ethyl acetate; 13:1) to give compound **I$_5$** as a white solid, with a yield of 56.8%. m.p. 83.4–84.6°C.

4.2.14. 2-Formyl-3-oxoursolic acid methyl ester (*II*$_1$)

To a solution of compound **2a** (50.0 mg, 0.107 mmol) in dichloromethane was added Vilsmeier reagent (freshly prepared) in an ice bath, and the mixture was stirred for 3 h at room temperature. Then a small amount of solid sodium acetate was added with stirring. The reaction end was monitored by TLC. The obtained reaction was treated with 0.3% $NaHCO_3$ to pH 7 and extracted with ethyl acetate, dried with anhydrous magnesium sulfate, and the solvent was distilled under reduced pressure to give the crude product which was purified on a silica gel column with petroleum ether–ethyl acetate (15:1) to give a white solid **II$_1$**, with a yield of 52.7%, m.p. 163.7–165.2°C.

4.2.15. 2-Formyl-3-oxoursolic acid ethyl ester (*II*$_2$)

According to the same method for compound **II$_1$**, compound **II$_2$** was prepared from **2b** (50.0 mg, 0.062 mmol). The crude product was purified by column chromatography (petroleum ether–ethyl acetate; 10:1) to give compound **II$_2$** as a white solid, with a yield of 56.2%, m.p. 175.1–177.5°C.

4.2.16. 2-Formyl-3-oxoursolic acid isopropyl ester (*II*$_3$)

According to the same method for compound **II$_1$**, compound **II$_3$** was prepared from **2c** (50.0 mg, 0.101 mmol). The crude product was purified by column chromatography (petroleum ether–ethyl acetate; 10:1) to give compound **II$_3$** as a white solid, with a yield of 45.6%. m.p. 150.5–152.3°C.

4.2.17. 2-Formyl-3-oxoursolic acid morpholine amide (*II*$_4$)

According to the same method for compound **II$_1$**, compound **II$_4$** was prepared from **2d** (50.0 mg, 0.095 mmol). The crude product was purified by column chromatography (petroleum ether–ethyl acetate; 20:1) to give compound **II$_4$** as a white solid, with a yield of 40.3%. m.p. 101.3–102.6°C.

4.2.18. 2-Formyl-3-oxoursolic acid aniline (*II*$_5$)

According to the same method for compound **II$_1$**, compound **II$_5$** was prepared from **2e** (50.0 mg, 0.094 mmol). The crude product was purified by column chromatography (petroleum ether–ethyl acetate; 15:1) to give compound **II$_5$** as a white solid, with a yield of 38.7%. m.p. 112.5–114.3°C.

4.2.19. 2-Formyl-3-oxoursolic acid propyl ester (*II*$_6$)

According to the same method for compound **II$_1$**, compound **II$_6$** was prepared from **2f** (50.0 mg, 0.101 mmol). The crude product was purified by column chromatography (petroleum ether–ethyl acetate; 15:1) to give compound **II$_6$** as a white solid, with a yield of 50.1%. m.p. 137.1–139.5°C.

4.3. Antitumor bioassays

Listed anticancer drug gefitinib was used as the positive control medicine; the antitumor activities of the 11 target compounds were evaluated by MTT assay in tumor cells including human cervical cancer cells (HeLa), human hepatoma cells (HepG2), and human gastric carcinoma cells (BGC-823). Logarithmic growth phase tumor cells were cultured in 96-well culture plate, each well 100 μl (containing 4000 cells approximately), and 10^{-5} moll^{-1} of test substance was added to the control group. To the negative control group and the treatment group was added an equal volume of solvent, cultured in an incubator

at 37°C, 5% CO_2 for 48 h. The culture fluid was discarded, MTT (1 mg ml^{-1}) 50 µl was added into each well 4 h before the end of the experiment. The supernatant was discarded and 150 µl DMSO was added to each well to dissolve formazan particles, then mildly shaken to dissolve. The absorption (OD) for each well was measured with enzyme labeling photometer at 490 nm wavelength and the inhibition rate of the cells for each drug was calculated. Each experiment was repeated at least three times and the results averaged.

Disclosure statement

No potential conflict of interest was reported by the authors.

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