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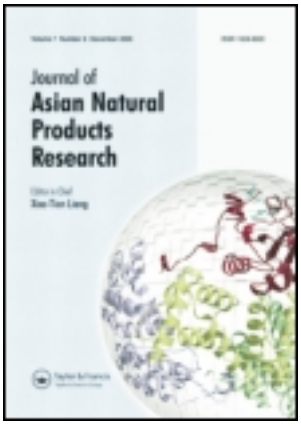

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Synthesis and biological evaluation of oleanolic acid derivatives as antitumor agents

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Synthesis and biological evaluation of oleanolic acid derivatives as antitumor agents

Lei Chen[a], Jian-Bo Wu[a], Fan Lei[a], Shan Qian[b], Li Hai[a] and Yong Wu[a]*

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Derivatives of oleanolic acid were synthesized and evaluated *in vitro* for their growth inhibition against human hepatocellular carcinoma cell line (HepG2) and colon cancer cell line (Col-02). Several derivatives exhibited moderate-to-good inhibitory activity, with **3** displaying the most promising inhibition [$GI_{50} = 1.75\ \mu M$ (HepG2), $0.71\ \mu M$ (Col-02)]. Structure–activity relationship analyses of these derivatives demonstrated that a 1-en-2-cyano-3-oxo in ring A and a nitro at C-17 were important in retention of the inhibition against HepG2 and Col-02 cells.

Keywords: oleanolic acid; derivatives; synthesis; antitumor; inhibition ratio

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1. Introduction

Oleanane-type pentacyclic triterpenoids are abundant in plant kingdom [1,2]. Oleanolic acid (3β-hydroxy-olea-12-en-28-oic acid, OA, **1**) was firstly separated from Oleaeurepaea, and exists widely in almost 190 species of 60 branches of food, medicinal herbs, and other plants in the form of free acid or aglycones for triterpenoid saponins [3]. It has been investigated for anti-inflammatory activity [4], protection of the liver against toxic injury [5], induction of collagen synthesis [6], and induction of differentiation in leukemia or teratocarcinoma cells [7].

In recent years, it was found that OA had marked antitumor effects and exhibited cytotoxic activity toward many cancer cell lines [6,8–14]. For example, OA has been demonstrated to have antihepatocellular carcinoma activity in humans without apparent side effects [8]. For the good activities, its modification becomes very hot; meanwhile, some sequent findings show very inspirited. For instance, 3-oxo-OA was found to significantly inhibit the growth of cancer cells derived from different tissues, and had inhibitory effect on melanoma *in vivo* [9], in which 2-cyano-3,12-dioxooleana-1,9(11)-dien-28-oic acid (CDDO) is the most prominent acid without any doubt. CDDO [15] showed high inhibitory activity against production of nitric oxide (NO) induced by interferon-γ in mouse macrophages ($IC_{50} = 0.1$ nM level) and also inhibited proliferation. Moreover, the analogs of CDDO even showed more potency than their parent compound [16,17]. CDDO and CDDO-methyl ester are currently in clinical trial for the treatment of cancers, which suggests that CDDOs have the potential to be a viable therapy.

During the structural modification of OA, we found that nitroethylene (vinyl) derivatives (**2**) showed strong inhibition

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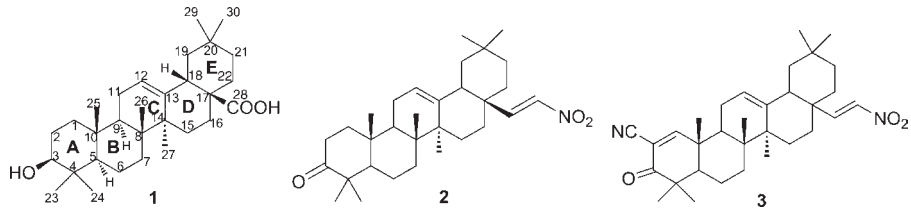

Figure 1. The chemical structures of compounds **1**–**3**.

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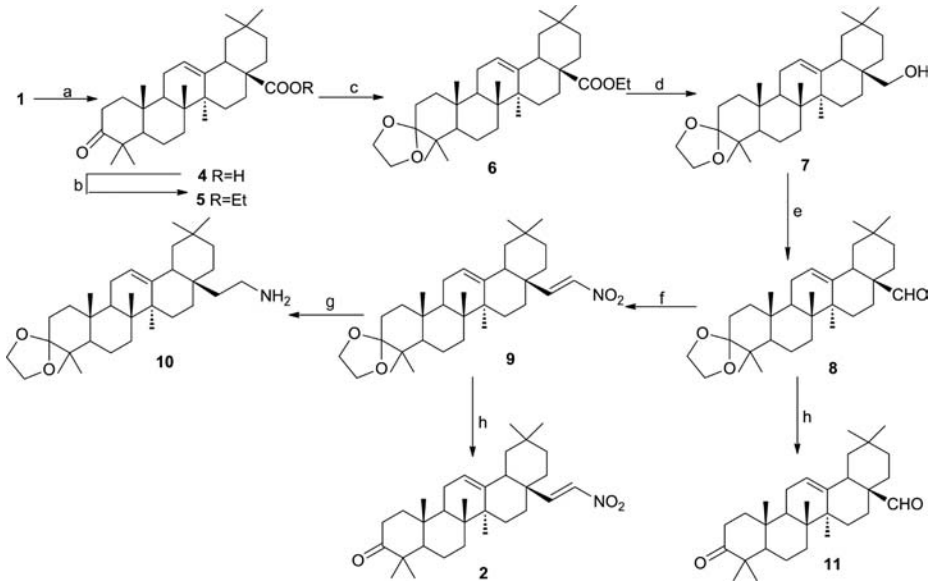
against HepG2 and Col-02 *in vitro* unexpectedly. Encouraged from these findings, we designed, synthesized serials of novel OA derivatives, and also evaluated their growth inhibitions against human hepatocellular carcinoma cell line (HepG2) and colon cancer cell line (Col-02), which are related to the most frequent cancers in the world [18]. On the design of the OA derivatives, we retained the nitroethylene at C-17 and combined it with the 1-en-2-cyano-3-oxo in ring A as CDDOs, excepting that the two groups could function in coordination with each other. Finally, 15 oleanane derivatives were synthesized and the evaluation result showed that several derivatives exhibited moderate-to-good inhibitory activity, with **3** displaying the best activity [$GI_{50} = 1.75\,\mu M$ (HepG2), $0.71\,\mu M$ (Col-02)]. We have also discussed the potential structure–activity relationship (SAR) of these compounds and the implication of these findings (Figure 1).

2. Results and discussion

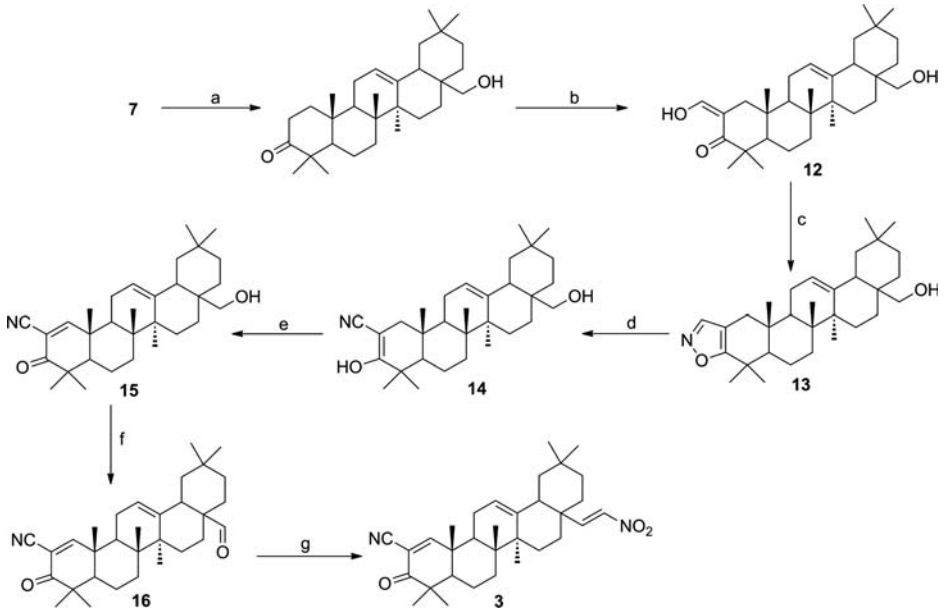
First, the modifications on C-17 of OA were carried out. Jone's oxidation of **1** furnished ketone **4**, which was reacted with bromoethane and K_2CO_3 in dry DMF to give ethyl oleanate **5** in a high yield. Acetalization of **5** with ethylene glycol gave **6**, followed by the reduction in $LiAlH_4$ afford to alcohol **7** [19]. Oxidation of **7** with pyridinium chlorochromate (PCC) afforded aldehyde **8**, which was then converted to **9** with CH_3COONH_4 and CH_3NO_2. Compound **9** was reduced to **10** using $LiAlH_4$. Deprotection of **6** and **7** yielded ketones **2** and **11**, respectively (Scheme 1). The ^1H NMR spectral data of ketone **2** displayed three olefinic hydrogens at δ 7.20, 6.93, and 5.35, respectively. The IR spectrum showed the olefinic hydrogen absorption bands at 3110, 2949, and $1524\,cm^{-1}$, suggesting the formation of nitrovinyl group. Elemental analysis and ESI-MS confirmed the structure of ketones **2** and **11**.

Subsequently, we designed a variety of new OA derivatives with modified ring A. Similarly, deprotection of ketal **7** yielded the ketone compound. Hydroxymethylene **12** was prepared by the formulation of the ketone compound with ethyl formate in the presence of sodium methoxide in benzene. Isoxazole **13** was prepared by the condensation of **12** with hydroxylamine. Cleavage of the isoxazole moiety of **13** with sodium methoxide gave nitrile **14**. Enone **15** was prepared by 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) oxidation of **14** in benzene [20]. Similarly, oxidation of **15** with PCC furnished aldehyde **16**, which was converted to **3** with CH_3COONH_4 and CH_3NO_2 (Scheme 2).

The inhibitions against HepG2 and Col-02 of OA (**1**) and its synthetic derivatives were evaluated *in vitro* (Table 1). The keto derivative **4** is a little more active than **1**. Acetylation of the C-3 keto group (**6**–**8**) diminished the inhibitory activities compared to **4**, indicating that C-3 keto groups contributed to inhibition. Introducing nitro group at C-17 of OA increased the potency by three times compared to **1** [**2**: $GI_{50} = 1.30\,\mu M$ (HepG2), $4.18\,\mu M$ (Col-02)]. These results indicated that the nitro moiety was important for the retention



Scheme 1. Synthesis of **2** and **4**–**11**. Reagents and conditions: (a) PCC, CH_2Cl_2, r.t.; (b) EtBr, K_2CO_3, DMF, r.t.; (c) $HOCH_2CH_2OH$, TsOH, toluene, ref.; (d) $LiAlH_4$, THF, r.t.; (e) PCC, CH_2Cl_2, r.t.; (f) CH_3COONH_4, CH_3NO_2, toluene, 70°C; (g) $LiAlH_4$, THF, r.t.; and (h) 2 mol/l dilute hydrochloric acid, THF, 100°C.



Scheme 2. Synthesis of **3** and **12**–**16**. Reagents and conditions: (a) 2 mol/l dilute hydrochloric acid, THF, 100°C; (b) $HCOOCH_2CH_3$, CH_3ONa, toluene, r.t.; (c) $NH_2OH.HCl$, EtOH/Ether, ref.; (d) CH_3ONa, Ether/MeOH, r.t.; (e) DDQ, toluene, ref.; (f) PCC, DCM, i.b.; and (g) CH_3NO_2, NH_4OAc, toluene, ref.

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Table 1. Growth inhibition rates and GI_{50} values of compounds **1**–**16** against human hepatocellular carcinoma and colon cancer cell lines.

Compounds	HepG2		Col-02	
	Growth %[a]	GI_{50} (μg/ml)	Growth %	GI_{50} (μg/ml)
1	104.82	ND	93.20	ND
2	33.78	1.30	−42.78	4.18
3	5.89	1.75	−12.63	0.71
4	73.11	ND	71.34	ND
5	134.94	ND	92.76	ND
6	124.66	ND	105.04	ND
7	133.87	ND	124.71	ND
8	139.57	ND	76.19	ND
9	50.39	ND	59.03	ND
10	96.73	ND	108.68	ND
11	90.55	ND	111.37	ND
12	73.00	ND	10.36	9.16
13	46.92	9.71	62.17	ND
14	105.58	ND	61.95	ND
15	50.79	ND	45.96	ND
16	27.42	5.31	3.46	1.44
Taxol[b]	5.36	0.05	28.28	0.03

Note: ND, not determined.
[a] The maximum inhibition concentration is 10 μg/ml.
[b] Taxol was used as the positive control, of which the maximum inhibition concentration is 10 μg/ml.

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of inhibitory activities. Several OA nitro derivatives (**2**, **3**, **9**) with modified ring A exhibited moderate-to-good inhibitory activity, with **3** displaying the best activity [GI_{50} = 1.75 μM (HepG2), 0.71 μM (Col-02)]. SAR analyses of these derivatives indicated that 1-en-2-cyano-3-oxo in ring A and a nitro at C-17 produced inhibition in coordination with each other as anticipated. Both the structures were important in the retention of inhibition against HepG2 and Col-02.

In summary, 15 oleanolic derivatives with modified ring A and substitutions at C-17 were synthesized and biologically evaluated against HepG2 and Col-02. Among the three series of compounds, **3** [GI_{50} = 1.75 μM (HepG2), 0.71 μM (Col-02)] exhibited more potent antitumor activity than the parent compound **1**. SAR analysis showed that a 1-en-2-cyano-3-oxo in ring A and a nitro at C-17 were important in retention of the inhibition against HepG2 and Col-02. Based on these results, further design and biological evaluation of OA derivatives as promising drugs are ongoing in our laboratories, and the results will be reported in due course.

3. Experimental

3.1 General experimental procedures

Melting points were measured on a YRT-3 melting point apparatus (Tianda Tianfa Technology Co., Ltd, Tianjin, China) and are uncorrected. Optical rotations were determined on a PerkinElmer model 241 polarimeter (PerkinElmer, Inc., CA, USA). IR spectra were obtained on a PerkinElmer 983 (PerkinElmer, Inc., CA, USA). Elemental analyses were carried out by Atlantic Microlab (Atlanta, GA, USA). NMR spectra were recorded on a Varian INOVA 400 (Varian, Palo Alto, CA, USA) and on a Bruker AC-E200. Chemical shifts (*d*) are expressed in ppm, tetramethylsilane used as the internal reference, and coupling constants (*J*) are expressed in Hz. Mass spectra were recorded on an Agilent 1946B ESI-MS instrument (Agilent Technologies, Inc.,

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Santa Clara, CA, USA). TLC was performed using precoated silica gel GF254 (0.2 mm; Chemical Industry Institute, Yantai, China), and column chromatography was performed using silica gel (100–200 mesh; Qingdao Haiyang Chemical Co., Ltd, Qingdao, China).

Oleanic acid (**1**) was purchased from Huakang Chemical Company, Inc. (Sichuan, China). HepG2 and Col-02 were supplied by Di Ao Pharmaceutical Group (Chengdu, SiChuan, China). Positive control Taxol was supplied by Shanghai Natural Bio-engineering Co. Ltd. (Shanghai, China).

3.2 Synthesis of compounds 2, 3, and 8–16

*3.2.1 Olean-12-en-28-aldehyde-3-spiro[1′, 2′]dioxolane (**8**)*

To a solution of **7** [18] (2.0 g, 4.0 mmol) in CH_2Cl_2 (12 ml) was added PCC (2.6 g, 12 mmol). The mixture was stirred at room temperature for 4 h. The solution was washed with water, dried over sodium sulfate, filtered, and concentrated, and the resulting residue was subjected to a silica gel column chromatography [petroleum ether (PE):EtOAc = 5:1] to give **8** (1.4 g, 75%) as a white solid. M.p.: 242–244°C; $[\alpha]_D^{20}$ +36.3 ($c = 0.5$, CH_2Cl_2); IR (KBr) υ_{max} 2950, 2862, 2801, 2701, 1724, 1462, 1383, 1202, 1111 cm^{-1}; ^1H NMR (CD_3Cl, 400 MHz): δ 9.40 (1H, s, H-28), 5.33 (1H, t, $J = 3.6$ Hz, H-12), 3.94 (4H, m, —OCH_2CH_2O—), 2.62 (1H, dd, $J = 14.0$, 4.4 Hz, H-18), 1.14 (3H, s, H-26), 0.95 (3H, s, H-27), 0.93 (3H, s, H-25), 0.92, 0.91, 0.85, 0.73 (each 3H, 12H, s, H-23, H-24, H-29, H-30); ESI-MS: *m/z* 483 $[M + H]^+$; Elemental analysis: Found: C, 79.76%; H, 10.29%; calcd for $C_{32}H_{50}O_4$: C, 79.62%; H, 10.44%.

*3.2.2 Olean-12-en-28-(2′-nitro-vinyl-)-3-spiro [1′, 2′]dioxolane (**9**)*

To a solution of **8** (1.6 g, 3.4 mmol) and CH_3COONH_4 (1.5 g, 19.5 mmol) in CH_3NO_2 (150 ml) was added toluene (2 ml). The mixture was stirred at 70°C for overnight and concentrated to remove CH_3NO_2. The residue was dispersed in CH_2Cl_2 and washed with aqueous $NaHCO_3$ and water and dried over sodium sulfate, filtered, and concentrated to afford yellow solid, and the resulting residue was subjected to a silica gel column chromatography (PE:EtOAc = 80:1) to give **9** (1.2 g, 69%) as a white solid. M.p.: 140–142°C; $[\alpha]_D^{20}$ +46.8 ($c = 0.5$, CH_2Cl_2); IR (KBr) υ_{max} 3112, 2950, 2866, 1640, 1525, 1462, 1383, 1346, 1239, 1113 cm^{-1}; ^1H NMR (CD_3Cl, 400 MHz) δ 7.20 (1H, d, $J = 13.6$ Hz, H-28), 6.94 (1H, d, $J = 13.6$ Hz, H-29), 5.35 (1H, t, $J = 3.6$ Hz, H-12), 3.94 (4H, m, —OCH_2CH_2O—), 2.33 (1H, dt, $J = 13.6$, 4.0 Hz, H-18), 2.22 (2H, dt, $J = 12.8$, 4.0 Hz, H-11), 1.14 (3H, s, H-26), 0.95 (3H, s, H-27), 0.93 (3H, s, H-25), 0.92, 0.91, 0.85, 0.73 (each 3H, 12H, s, H-23, H-24, H-30, H-31); ESI-MS: *m/z* 526 $[M + H]^+$; Elemental analysis: Found: C, 75.47%; H, 9.88%; N, 2.54%; calcd for $C_{33}H_{51}NO_4$: C, 75.39%; H, 9.78%; N, 2.66%.

*3.2.3 Olean-12-en-28-(2′-amino-ethyl)-3-spiro[1′, 2′]dioxolane (**10**)*

To a suspension of $LiAlH_4$ (0.5 g, 12.7 mmol) in absolute THF (10 ml) was added the solution of **9** (2.0 g, 2.8 mmol) in THF (10 ml) dropwise. The mixture was stirred at room temperature overnight to remove THF. The resulting residue was subjected to a silica gel column chromatography (PE:EtOAc = 5:1) to give **10** (779.3 mg, 56%) as a white solid. M.p.: 78–80°C; $[\alpha]_D^{20}$ +27.7 ($c = 0.5$, CH_2Cl_2); IR (KBr) υ_{max} 3445, 2924, 2862, 1463, 1382, 1202, 1109, 1057 cm^{-1}; ^1H NMR (CD_3Cl, 400 MHz): δ 5.15 (1H, t, $J =$ 3.2 Hz, H-12), 3.92 (4H, m, —OCH_2CH_2-O—), 3.58 (2H, q, $J = 7.2$ Hz, H-29), 2.71 (1H, m, H-18), 2.54 (2H, br s, —NH_2), 2.53 (2H, m, H-11), 1.83 (2H, m, H-28), 1.09(3H, s, H-26), 0.96(3H, s, H-27), 0.95 (3H, s, H-25), 0.91, 0.84 (each 3H, 6H, s,

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H-23, H-24); ESI-MS: *m/z* 498 $[M + H]^+$; Elemental analysis: Found: C, 79.49%; H, 11.29%; N, 2.78%; calcd for $C_{33}H_{55}NO_2$: C, 79.62%; H, 11.14%; N, 2.81%.

*3.2.4 3-Oxo-12-en-28-(2′-nitro-vinyl)-olean (**2**)*

According to the reported method [19], compound **2** was obtained as a white solid from dioxolane **9**, yield 78%. M.p.: 98–100°C; $[\alpha]_D^{20}$ +87.3 ($c = 0.5$, CH_2Cl_2); IR (KBr) υ_{max} 3110, 2949, 2866, 1704, 1641, 1524, 1462, 1347, 1256 cm^{-1}. ^1H NMR (CD_3Cl, 400 MHz): δ 7.20 (1H, d, $J = 14.0$ Hz, H-28), 6.93 (1H, d, $J = 14.0$ Hz, H-29), 5.35 (1H, t, $J = 3.6$ Hz, H-12), 2.57 (1H, m, H-18), 2.36 (2H, m, H-11), 1.16 (3H, s, H-26), 1.09 (3H, s, H-27), 1.06 (3H, s, H-25), 1.05, 0.93, 0.90, 0.84 (each 3H, 12H, s, H-23, H-24, H-30, H-31); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 217.5 (C, C-3), 151.9 (CH, C-28), 143.2 (C, C-13), 139.7 (CH, =$CHNO_2$), 123.3 (CH, C-12), 55.3 (C, C-17), 47.5 (CH, C-5), 46.8 (C, C-4), 46.0 (CH, C-9), 43.6 (CH_2, C-19), 41.8 (CH, C-18), 39.6 (C, C-14), 39.2 (CH, C-1), 39.0 (C, C-8), 36.7 (C, C-10), 34.1 (CH_2, C-21), 34.0 (CH_2, C-22), 33.7 (C, C-2), 32.9 (CH_2, C-7), 31.9 (CH_2, C-15), 30.7 (C, C-20), 27.1 (CH_2, C-16), 26.3 (CH_3, C-30), 26.1 (CH_3, C-29), 25.8 (CH_3, C-27), 23.6 (CH_3, C-24), 23.4 (CH_3, C-23), 21.5 (CH_2, C-11), 19.3 (CH_2, C-6), 16.7 (CH_3, C-26), 15.0 (CH_3, C-25); ESI-MS: *m/z* 482 $[M + H]^+$; Elemental analysis: Found: C, 77.36%; H, 9.86%; N, 2.85%; calcd for $C_{31}H_{47}NO_3$: C, 77.29%; H, 9.83%; N, 2.91%.

Compound **11** was prepared according to the same procedure described for **2**. M.p.: 153–155°C; $[\alpha]_D^{20}$ +88.8 ($c = 0.5$, CH_2Cl_2); IR (KBr) υ_{max} 2950, 2928, 2859, 2707, 1708, 1459, 1382, 1160, 1113 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 9.40 (1H, s, H-28), 7.73 (1H, s, H-1), 5.37 (1H, t, $J = 3.6$ Hz, H-12), 2.63 (1H, dd, $J = 13.2$, 4.0 Hz, H-18), 2.55 (1H, dt, $J = 16.0$, 3.6 Hz, H-2), 2.36 (1H, dq, $J = 16.0$, 3.6 Hz, H-2′), 1.85–2.03 (4H, m, H-11, H-22), 1.15 (3H, s, H-26), 1.09 (3H, s, H-27), 1.04 (3H, s, H-25), 1.04, 0.92, 0.92, 0.80 (each 3H, 12H, s, H-23, H-24, H-29, H-30).

*3.2.5 2-Hydroxymethylene-3-oxoolean-12(13)-en-28-ol (**12**)*

A solution of **7** (5.76 g, 12.0 mmol) in 2 M HCl (60 ml) and THF (100 ml) was refluxed for 1 h, and then cooled to room temperature. The organic layer was separated and the aqueous layer was extracted with EtOAc. The combined organic layer was washed with brine, dried over Na_2SO_4, condensed to give an amorphous solid. Without any purification, the solid and NaOMe (6.43 g, 119 mmol) were dissolved in dry toluene (150 ml) and stirred at room temperature for 10 min. Then ethyl formate (8.28 g, 119 mmol) was dropped into the solution, which was stirred at room temperature for 3 h. The mixture was diluted with toluene, washed by turns with 5% aqueous HCl solution, saturated aqueous $NaHCO_3$ solution and brine, dried over Na_2SO_4, and filtered. The filtrate was evaporated *in vacuo* to give a brown oil, which was purified by flash column chromatography (PE:EtOAc = 40:1) to give **12** (4.25 g, 76.20%) as an amorphous solid. M.p.: 86–88°C; $[\alpha]_D^{20}$ +96.0 ($c = 0.5$, CH_2Cl_2); IR (KBr) υ_{max} 3426, 2947, 2924, 2864, 1634, 1584, 1460, 1362, 1051 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 14.92 (1H, s, HO-C =), 8.58 (1H, s, H-C = O), 5.25 (1H, t, $J = 3.6$ Hz, H-12), 3.57 (1H, d, $J = 10.8$ Hz, CH_2OH), 3.23 (1H, d, $J = 10.8$ Hz, CH_2OH), 2.29 (2H, dd, $J = 14.4$, 3.2 Hz, H-1), 1.20 (3H, s, H-26), 1.19 (3H, s, H-27), 1.13 (3H, s, H-25), 1.00, 0.93, 0.90, 0.89 (each 3H, s, H-23, H-24, H-29, H-30); ESI-MS: *m/z* 469 $[M + H]^+$; Elemental analysis: Found: C, 79.31%; H, 10.26%; calcd for $C_{31}H_{48}O_3$: C, 79.44%; H, 10.32%.

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3.2.6 Isoxazolo[4, 5-b]olean-12(13)-en-28-ol (**13**)

A solution of **12** (150 mg, 0.32 mmol) in EtOH (10 ml) and water (4 ml) was added hydroxylamine hydrochloride (222.37 mg, 3.2 mmol). The mixture was heated under reflux for 1 h and concentrated. Water was added and the mixture was extracted with EtOAc. The combined organic layers were washed with water and brine twice, respectively, dried over Na_2SO_4 and filtered. The filtrate was evaporated *in vacuo* to give a solid, which was purified by flash column chromatography (PE:EtOAc = 30:1) to give **13** (110 mg, 73.81%) as an amorphous solid. M.p.: 81–83°C; $[\alpha]_D^{20}$ +91.1 (c = 0.5, CH_2Cl_2); IR (KBr) υ_{max} 3425, 2947, 2864, 1640, 1482, 1460, 1382, 1367, 1050 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.99 (1H, s, H-C = N), 5.26 (1H, t, J = 3.6 Hz, H-12), 3.57 (1H, d, J = 10.8 Hz, CH_2OH), 3.24 (1H, d, J = 10.8 Hz, CH_2OH), 1.32 (3H, s, H-26), 1.24 (3H, s, H-27), 1.19 (3H, s, H-25), 1.00, 0.91, 0.90, 0.89 (each 3H, s, H-23, H-24, H-29, H-30); ESI-MS: m/z 466 $[M + H]^+$; Elemental analysis: Found: C, 79.89%; H, 10.23%; N, 3.09%; calcd for $C_{31}H_{47}NO_2$: C, 79.95%; H, 10.17%; N, 3.01%.

3.2.7 2-Cyano-3-oxoolean-12(13)-en-28-ol (**14**)

A solution of **13** (100 mg, 0.215 mmol) in MeOH (4 ml) and Et_2O (8 ml) in an ice bath was added NaOMe (46.44 g, 0.86 mmol). The mixture was stirred at room temperature for 6 h, and then concentrated. Water was added and the mixture was extracted with DCM. The combined organic layers were washed by turns with 5% aqueous HCl solution, saturated aqueous $NaHCO_3$ solution and brine, dried with Na_2SO_4, and filtered. The filtrate was evaporated *in vacuo* to give a solid, which was purified by flash column chromatography (PE:EtOAc = 20:1) to give **14** (85 mg, 85.00%) as a white solid. M.p.: 150–152°C; $[\alpha]_D^{20}$ +101.8 (c = 0.5, CH_2Cl_2); IR (KBr) υ_{max} 3422, 2948, 2926, 2866, 2206, 1718, 1627, 1460, 1382, 1367, 1267, 1047 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 5.88 (1H, OH-3), 5.23 (1H, t, J = 3.6 Hz, H-12), 3.56 (1H, d, J = 10.8 Hz, CH_2OH), 3.22 (1H, d, J = 10.8 Hz, CH_2OH), 1.26 (3H, s, H-26), 1.17 (3H, s, H-27), 1.08 (3H, s, H-25), 0.98, 0.97, 0.89, 0.88 (each 3H, s, H-23, H-24, H-29, H-30); ESI-MS: m/z 466 $[M + H]^+$; Elemental analysis: Found: C, 79.87%; H, 10.13%; N, 3.06%; calcd for $C_{31}H_{47}NO_2$: C, 79.95%; H, 10.17%; N, 3.01%.

3.2.8 2-Cyano-3-oxoolean-1, 12(13)-dien-28-ol (**15**)

A mixture of **14** (600 mg, 1.29 mmol) and DDQ (439.26 mg, 1.95 mmol) in dry toluene (50 ml) was heated under reflux for 8 h and filtered. The insoluble matter was washed with EtOAc and the combined organic layers were washed with 5% aqueous NaOH solution and brine, respectively, dried over Na_2SO_4, and filtered. The filtrate was evaporated *in vacuo* to give a dark oil, which was purified by flash column chromatography (PE:EtOAc = 10:1) to give **15** (447.5 mg, 74.82%) as a light yellow amorphous solid. M.p.: 161–162°C; $[\alpha]_D^{20}$ +76.1 (c = 0.5, CH_2Cl_2); IR (KBr) υ_{max} 3447, 2949, 2868, 2235, 1687, 1461, 1385, 1224, 1050 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.75 (1H, s, H-1), 5.36 (1H, t, J = 3.6 Hz, H-12), 3.57 (1H, d, J = 10.8 Hz, CH_2OH), 3.21 (1H, d, J = 10.8 Hz, CH_2OH), 1.24 (3H, s, H-26), 1.22 (3H, s, H-27), 1.19 (3H, s, H-25), 1.15, 1.05, 0.91, 0.89 (each 3H, s, H-23, H-24, H-29, H-30); ESI-MS: m/z 464 $[M + H]^+$; Elemental analysis: Found: C, 80.41%; H, 9.83%; N, 3.05%; calcd for $C_{31}H_{45}NO_2$: C, 80.30%; H, 9.78%; N, 3.02%.

3.2.9 2-Cyano-3-oxoolean-1, 12(13)-dien-28-aldehyde (**16**)

A solution of **15** (792.1 mg, 1.71 mmol) in DCM (30 ml) was dropped into the

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solution of PCC (735.3 mg, 3.42 mmol) in DCM (30 ml) and stirred at room temperature for 0.5 h and filtered. The insoluble matter was washed with DCM. The combined organic layers were washed with brine twice, dried over Na_2SO_4, and filtered. The filtrate was evaporated *in vacuo* and purified by flash column chromatography (PE:EtOAc = 60:1) to give **16** (410 mg, 51.93%) as a white solid. M.p. >200°C (decomp); $[\alpha]_D^{20}$ +58.5 ($c = 0.5$, CH_2Cl_2); IR (KBr) υ_{max} 2922, 2856, 2697, 2234, 1725, 1691, 1464, 1383, 1220, 1005 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 9.39 (1H, s, H-28), 7.73 (1H, s, H-1), 5.42 (1H, t, $J = 3.6$ Hz, H-12), 2.68 (1H, dd, $J = 13.8$, 4.4 Hz, H-18), 1.22 (3H, s, H-26), 1.21 (3H, s, H-27), 1.16 (3H, s, H-25), 1.14, 0.94, 0.93, 0.84 (each 3H, s, H-23, H-24, H-29, H-30); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 207.0 (C, C-3), 198.0 (CH, C-28), 170.0 (CH, C-1), 143.7 (C, C-13), 121.7 (CH, C-12), 114.9 (C, CN), 113.8 (C, C-2), 52.5 (C, C-17), 49.1 (CH, C-5), 45.2 (CH_2, C-19), 44.8 (C, C-14), 42.0 (C, C-4), 41.0 (CH, C-9), 40.5 (C, C-10), 40.5 (C, C-8), 40.3 (CH, C-18), 33.0 (CH_2, C-21), 32.1 (CH_2, C-7), 30.6 (C, C-20), 29.6 (CH_2, C-15), 27.7 (CH_2, C-22), 27.5 (CH_3, C-30), 26.5 (CH_3, C-29), 25.3 (CH_3, C-27), 23.3 (CH_2, C-11), 23.2 (CH_2, C-16), 21.8 (CH_3, C-24), 21.6 (CH_3, C-23), 18.6 (CH_2, C-6), 17.9 (CH_3, C-25), 17.5 (CH_3, C-26); ESI-MS: *m/z* 462 $[M + H]^+$; Elemental analysis: Found: C, 80.57%; H, 9.30%; N, 3.01%; calcd for $C_{31}H_{43}NO_2$: C, 80.65%; H, 9.39%; N, 3.03%.

3.2.10 2-Cyano-3-oxo-28-(β-nitro) vinylolean-1,12(13)-diene (3)

A solution of **16** (100 mg, 0.217 mmol) and NH_4OAc (333.5 mg, 4.34 mmol) in CH_3NO_2 (15 ml) and toluene (2 ml) was heated under reflux for 6 h and concentrated. Water was added and the mixture was extracted with DCM. The combined organic layers were washed with brine, dried over Na_2SO_4, and filtered. The filtrate was evaporated *in vacuo* and purified by flash column chromatography (PE:EtOAc = 40:1) to give **3** (52 mg, 47.50%) as an amorphous solid. M.p. >230°C (decomp); $[\alpha]_D^{20}$ +69.3 ($c = 0.5$, CH_2Cl_2); IR (KBr) υ_{max} 3112, 2949, 2923, 2857, 2230, 1689, 1520, 1464, 1382, 1349, 1014 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz): δ 7.73 (1H, s, H-1), 7.20 (1H, d, $J = 14.0$ Hz, H-28), 6.93 (1H, d, $J = 14.0$ Hz, H-29), 5.41 (1H, t, $J = 3.6$ Hz), 2.42 (1H, dd, $J = 13.8$, 4.4 Hz, H-18), 1.23 (3H, s, H-26), 1.21 (3H, s, H-27), 1.17 (3H, s, H-25), 1.14, 0.94, 0.91, 0.89 (each 3H, s, H-23, H-24, H-29, H-30); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 197.9 (C, C-3), 169.5 (CH, C-1), 151.6 (CH, C-28), 143.7 (C, C-13), 139.5 (CH, =CHNO$_2$), 122.0 (CH, C-12), 114.8 (C, CN), 113.8 (C, C-2), 52.3 (C, C-17), 45.7 (CH, C-5), 44.7 (C, C-14), 43.7 (CH_2, C-19), 42.0 (C, C-4), 41.0 (CH, C-18), 40.5 (C, C-10), 40.4 (C, C-8), 38.9 (CH, C-9), 33.8 (CH_2, C-21), 33.6 (CH_2, C-22), 32.8 (CH_2, C-7), 31.6 (CH_2, C-16), 30.6 (C, C-20), 29.6 (CH_2, C-15), 27.7 (CH_3, C-30), 26.8 (CH_3, C-29), 25.8 (CH_3, C-27), 25.6 (CH_2, C-11), 23.3 (CH_3, C-24), 23.2 (CH_3, C-23), 18.5 (CH_2, C-6), 17.7 (CH_3, C-25), 17.2 (CH_3, C-26); ESI-MS: *m/z* 505 $[M + H]^+$; Elemental analysis: Found: C, 76.23%; H, 8.82%; N, 5.51%; calcd for $C_{32}H_{44}NO_3$: C, 76.15%; H, 8.79%; N, 5.55%.

3.3 Cytotoxic assay in vitro

The cytotoxicity of individual compound screened was determined by the sulforhodamine B assay using the cytotoxicity detection kit (Roche Diagnostics, Shanghai, China) according to the manufacturer's instructions. The method described here has been optimized for the cytotoxic screening of compounds to adherent cell in a 96-well microtiter plate. After an incubation period, cell monolayers are fixed with 10% (w/v) trichloroacetic acid and stained for 30 min, after which the

excess dye is removed by washing repeatedly with 1% (v/v) acetic acid. The protein-bound dye is dissolved in 10 mM Tris base solution for oleanolic acid derivatives (OD) determination at 510 nm using a microplate reader. The percent specific cytotoxicity of each compound was determined based on [(OD experiments)−(OD negative controls)]/[(OD positive controls)−(OD negative controls)] × 100. The assay result was provided by the drug screening center of Di Ao Pharmaceutical Group.

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References

[1] N. Zhu, S. Sheng, S. Sang, J.W. Jhoo, N. Bai, and M.V. Karwe, *J. Agric. Food Chem.* **50**, 865 (2002).
[2] B.S. Siddiqui, I. Sultana, and S. Begum, *Phytochemistry* **54**, 861 (2000).
[3] R.K. Upadhyay, M.B. Pandey, R.N. Jha, V.P. Singh, and V.B. Pandey, *J. Asian Nat. Prod. Res.* **3**, 207 (2001).
[4] G.B. Singh, S. Singh, S. Bani, B.D. Gupta, and S.K. Banerjee, *J. Pharm. Pharmacol.* **44**, 456 (1992).
[5] Y.N. Zhang, W. Zhang, D. Hong, L. Shi, Q. Shen, J.Y. Li, J. Li, and L.H. Hua, *Bioorg. Med. Chem.* **16**, 8697 (2008).
[6] H.Y. Hsu, J.J. Yang, and C.C. Lin, *Cancer Lett.* **111**, 7 (1997).
[7] J. Liu, Y. Liu, and A.J. Parkinson, *Pharmacol. Exp. Ther.* **275**, 768 (1995).
[8] L. Chen, Y.H. Zhang, X.W. Kong, E. Lan, Z.J. Huang, S.X. Peng, D.L. Kaufman, and J. Tian, *J. Med. Chem.* **51**, 4834 (2008).
[9] D. Huang, Y. Ding, Y. Li, W.M. Zhang, W.S. Fang, and X.G. Chen, *Cancer Lett.* **233**, 289 (2006).
[10] C. Martin-Cordero, M. Reyes, M.J. Ayuso, and M.V. Toro, *Naturforschung* **56**, 45 (2001).
[11] B.S. Yun, I.J. Ryoo, I.K. Lee, K.H. Park, D.H. Choung, K.H. Han, and I.D. Yoo, *J. Nat. Prod.* **62**, 764 (1999).
[12] S. Taniguchi, Y. Imayoshi, E. Kobayashi, Y. Takamatsu, H. Ito, and T. Hatano, *Phytochemistry* **59**, 315 (2002).
[13] J. Liu, *J. Ethnopharmacol.* **49**, 57 (1995).
[14] J. Li, W.J. Guo, and Q.Y. Yang, *World J. Gastroenterol.* **8**, 493 (2002).
[15] T. Honda, B.V. Rounds, G.W. Gribble, N. Suh, Y. Wang, and M.B. Sporn, *Bioorg. Med. Chem. Lett.* **8**, 2711 (1998).
[16] T. Honda, Y. Honda, F.G. Favaloro Jr, G.W. Gribble, N. Suh, A.E. Place, M.H. Rendi, and M.B. Sporn, *Bioorg. Med. Chem. Lett.* **12**, 1027 (2002).
[17] A.E. Place, N. Suh, C.R. Williams, R. Risingsong, T. Honda, Y. Honda, G.W. Gribble, L.M. Leesnitzer, J.B. Stimmel, T.M. Willson, E. Rosen, and M.B. Sporn, *Clin. Cancer. Res.* **9**, 2798 (2003).
[18] D.M. Parkin, F. Bray, J. Ferlay, and P. Pisani, *Int. J. Cancer.* **94**, 153 (2001).
[19] S. Qian, H.J. Li, Y. Chen, W.Y. Zhang, S.Y. Yang, and Y. Wu, *J. Nat. Prod.* **73**, 1743 (2010).
[20] T. Honda, G.W. Gribble, N. Suh, H.J. Finlay, B.V. Rounds, L. Bore, F.G. Favaloro Jr, Y.P. Wang, and M.B. Sporn, *J. Med. Chem.* **43**, 1866 (2000).

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