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Synthesis and cytotoxicity evaluation of oleanolic acid derivatives

Jia Hao [a,b], Jun Liu [c], Xiaoan Wen [b,*], Hongbin Sun [b,*]

[a] Tianjin Key Laboratory of Phytochemistry and Pharmaceutical Analysis, Tianjin University of Traditional Chinese Medicine, Tianjin 300193, PR China
[b] State Key Laboratory of Natural Medicines, China Pharmaceutical University, 24 Tongjiaxiang, Nanjing 210009, PR China
[c] Jiangsu Center for Drug Screening, China Pharmaceutical University, 1 Shennonglu, Nanjing 210038, PR China

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ABSTRACT

Twelve derivatives of oleanolic acid (**1**) have been synthesized and evaluated for their inhibitory activities against the growth of prostate PC3, breast MCF-7, lung A549, and gastric BGC-823 cancer cells by MTT assays. Within these series of derivatives, compound **17** exhibited the most potent cytotoxicity against PC3 cell line (IC_{50} = 0.39 μM) and compound **28** displayed the best activity against A549 cell line (IC_{50} = 0.22 μM). SAR analysis indicates that H-donor substitution at C-3 position of oleanolic acid may be advantageous for improvement of cytotoxicity against PC3, A549 and MCF-7 cell lines.

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Triterpenoids, especially pentacyclic triterpenes, continuously attract great attention due to their amazing diversity of structures and biological activities. This is evidenced by the development of anti-HIV agent bevirimat, anti-cancer agent bardoxolone methyl (Fig. 1), immunological adjuvant QS-21 and anti-hepatitic drug diammonium glycyrrhizinate.[1–3] Oleanolic acid (**1**, OA), a naturally occurring pentacyclic triterpene acid widely distributed in food and medicinal plants, is one of the most popularly studied pentacyclic triterpenes. Its biological functions include anti-inflammation, anti-HIV, antioxidation, antidiabetes, hepatoprotection, and anti-cancer effects, etc.[4–8] It was shown that OA could suppress TPA-induced tumor promotion and exhibit direct cytotoxicity, proliferative inhibition or apoptotic effects in many cancer cell lines such as HCT15, A549, H460, Hep G2, Hep3B, Huh7, and HA22T, etc.[9–17] Mechanism studies on anti-multidrug resistance demonstrated that OA was effective to inhibit the activity of multidrug resistance protein ABCC1, but not the ABCB1,[18] suggesting that OA might be useful for both prevention and treatment of cancers. Commendably, OA presented low toxicity to normal cells[18] and its safety has been validated through over 20 years of clinical use for treatment of liver disorders in China.

As part of our efforts in developing pentacyclic triterpenes as therapeutic agents, we were in pursuit of novel anti-cancer agents based on OA. OA is constituted by a rigid pentacyclic sketelon,

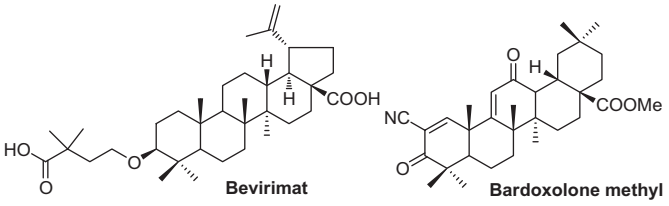

Figure 1. Structures of bevirimat and bardoxolone methyl.

which is highly hydrophobic and makes OA poorly water-soluble. Very recently, Biedermann et al. evaluated a series of quaternary ammonium salt derivatives of pentacyclic triterpene acids for their anti-cancer activities and pointed out that the cytotoxic activities of these compounds were correlated with their hydrophilicity.[19] Ma et al. investigated the cytotoxic activity of a series of oleanolic acid derivatives in Hep G2 cell line,[20] and their study results also suggested that lipophilicity was an important factor for cytotoxicity. In their study, 3β-amino-olean-12-en-28-oic acid methyl ester was identified to be highly cytotoxic to Hep G2 cells (IC_{50} = 4 μM). These raised our aspiration to search for drug-like anti-cancer agents through hydrophilic modifications of OA.

By long-term experience with pentacyclic triterpenes, we were aware that direct installation of common hydrophilic functions on the skeleton of OA might not greatly improve the whole molecular hydrophilicity. Although OA bears a hydroxyl group at C-3 position and a carboxy group at C-17 position, the contribution of these two

* Corresponding authors. Tel.: +86 25 83271198; fax: +86 25 83271043.
E-mail addresses: wxagj@126.com (X. Wen), hbsun2000@yahoo.com (H. Sun).

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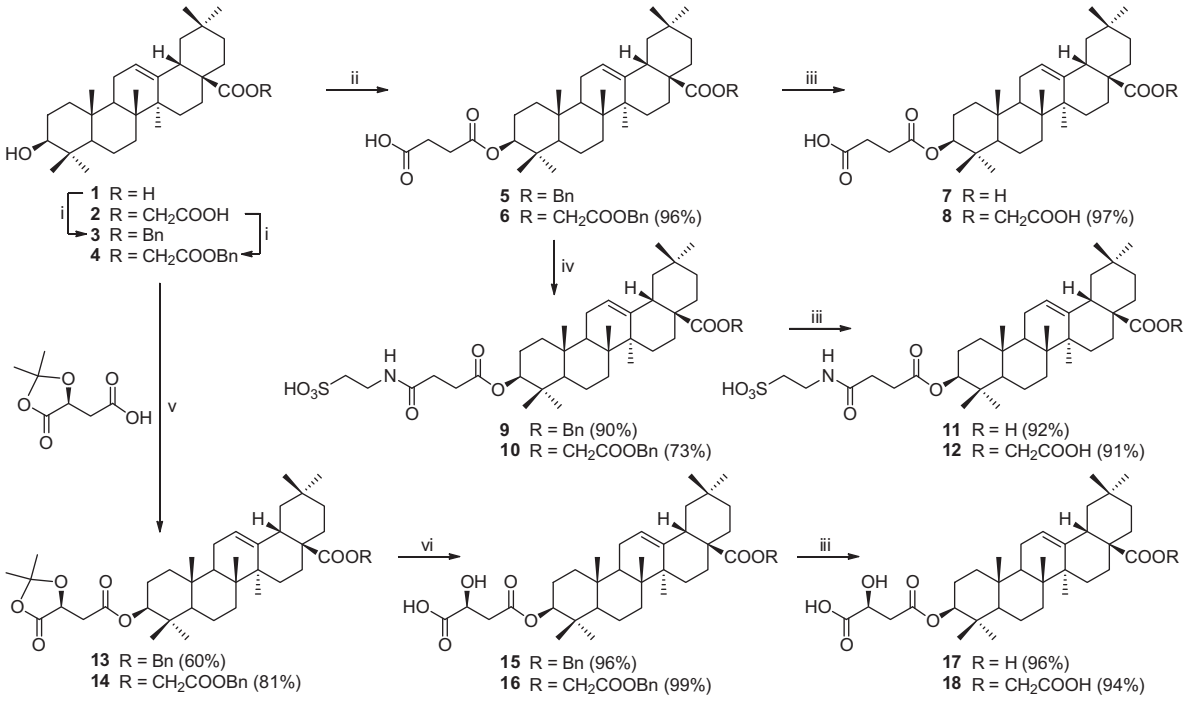
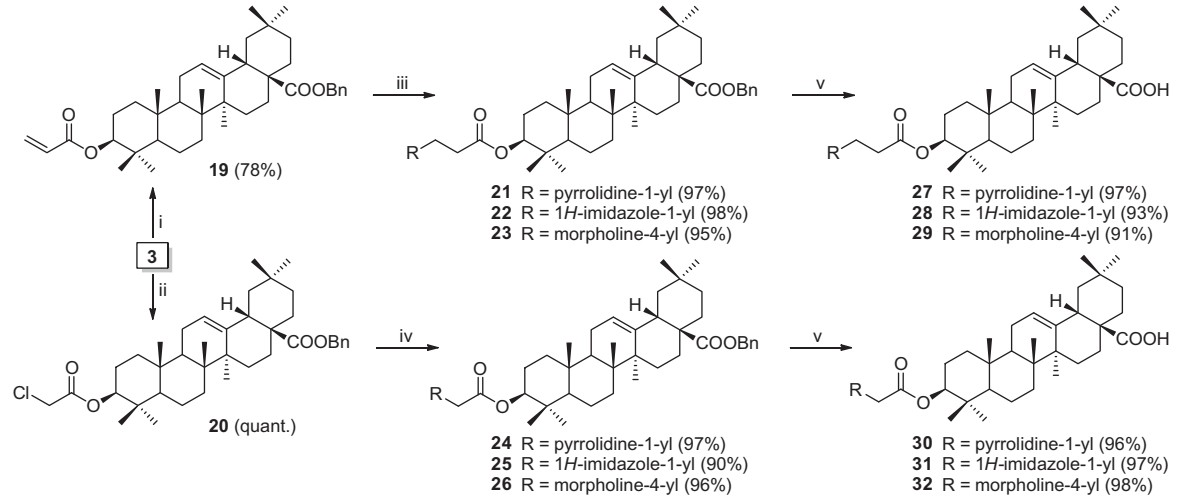


Scheme 1. Reagents and conditions: (i) BnCl/K_2CO_3/DMF; (ii) succinic anhydride/DMAP/pyridine; (iii) H_2/10% Pd–C/THF; (iv) *iso*-butyl chloroformate/Et_3N/THF, then taurine/Et_3N/CH_3CN; (v) DCC/DMAP/DCM; (vi) HAc/H_2O/THF.



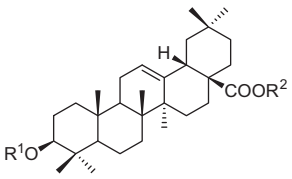
Scheme 2. Reagents and conditions: (i) acrylyl chloride/DMAP/DCM; (ii) chloroacetyl chloride/DMAP/THF; (iii) amine/Et_3N/DCM; (iv) amine/DMF; (v) H_2/10% Pd–C/THF.

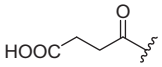
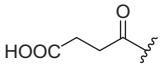
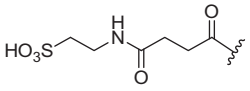
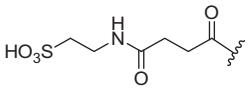
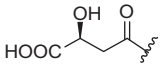
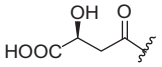
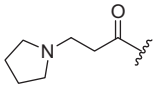
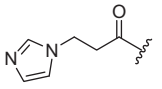
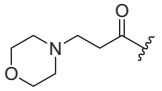
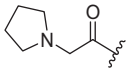
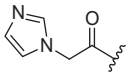
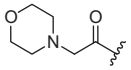
hydrophilic groups to the whole hydrophilicity of OA is limited given the large surface area of hydrophobic environment. We assumed that hydrophilic moieties coupled with certain long flexible spacers could contribute more to improve the hydrophilictiy of OA. With this in mind, we designed succinic acid esters **7** and **8**, taurine amides **11** and **12**, L-malic acid esters **17** and **18**, and N-heterocycles **27**–**32** (Schemes 1 and 2). Here, we report their syntheses and cytotoxicity evaluation in four cancer cell lines.

Synthesis of compounds **7**, **8**, **11**, **12**, **17** and **18** is outlined in Scheme 1. According to our previous studies,[21,22] oleanolic acid (**1**) was successively benzylated with benzyl chloride, acylated using succinic acid anhydride, and debenzylated by hydrogenolysis over Pd/C to give the known acid **7**. Upon treatment with *iso*-butyl chloroformate and taurine, carboxylic acid **5** from the above sequence was converted to amide **9**, which was further hydrogenolyzed to give acid **11**. In presence of DCC and DMAP, alcohol **3** was coupled with 2-[(4*S*)-2,2-dimethyl-5-oxo-1,3-dioxolan-4-yl]acetic acid to give ester **13**. Isopropylidene group of ester **13** was then removed in aqueous AcOH solution to supply compound **15**. Finally, hydrogenolysis of compound **15** afforded acid **17**. In similar fashions, compounds **8**, **12** and **18** were synthesized from carboxylic acid **2**, whose preparation was described in our previous report.[23]

Compounds **27**–**32** were prepared following the procedures in Scheme 2. In brief, alcohol **3** was acylated to acrylic acid ester **19** and chloroacetic acid ester **20**, respectively. Acrylic acid ester **19** underwent Michael addition reactions with pyrrolidine, imidazole and morpholine to afford the corresponding compounds **21**, **22** and **23** in more than 95% yields. Compounds **21**, **22** and **23** were further hydrogenolyzed to afford the final compounds **27**, **28** and **29**,

Table 1
IC_{50} values of the assayed compounds against the growth of PC3, A549, MCF-7 and BGC-823 cancer cells



Compound	R^1	R^2	IC_{50}[a] (μM)			
			PC3	A549	MCF-7	BGC-823
Adriamycin[b]	/	/	0.68	0.54	1.65	1.33
1[b]	H	H	6.51	0.39	35.4	2.59
7		H	7.11	6.57	60.7	5.59
8		CH_2COOH	7.72	11.9	805	13.4
11		H	0.83	1.31	5.19	7.32
12		CH_2COOH	10.4	268.1	51.7	84.5
17		H	0.39	0.71	16.2	4.18
18		CH_2COOH	8.07	19.3	35.1	9.36
27		H	5.45	6.12	1.98	27.2
28		H	50.5	0.22	NI[c]	76.3
29		H	6.38	727.5	718	81.9
30		H	28.3	8.53	12.9	87.2
31		H	57.5	16.9	894	16.3
32		H	6.29	8.27	14.9	24.5

[a] Each value represents the mean of three determinations.
[b] Positive control.
[c] NI means no inhibition.

respectively. Reaction of chloroacetic acid ester **20** with the above mentioned amines to give compounds **24**, **25** and **26**, respectively. Finally, they were also debenzylated to afford products **30**, **31** and **32**, respectively.

The target compounds **7**, **8**, **11**, **12**, **17**, **18** and **27**–**32** were subjected to evaluation for their inhibitory activity against the growth of PC3, MCF-7, A549, and BGC-823 cancer cells by MTT assays.[24] Adriamycin and oleanolic acid were used as positive controls in this assay. IC_{50} values are listed in Table 1.

As shown in Table 1, most of the 12 derivatives exhibited inhibitory activity against growth of the four cancer cell lines. Within theses series of compounds, imidazole **28** displayed the best activity in A549 cell line (IC_{50} = 0.22 μM) despite low or no activity in the other cells. The inhibitory activity of malic acid ester **17** against the growth of PC3 cells (IC_{50} = 0.39 μM) was 1.7 times more potent than that of adriamicin (IC_{50} = 0.68 μM) and near 17-fold more potent than that of OA (IC_{50} = 6.51 μM). In the same cell line, taurine amide **11** showed sevenfold more potent activity (IC_{50} = 0.83 μM) than OA and almost comparatable activity to adriamicin. In MCF-7 cell line, the cytotoxic activity of pyrrolidine compound **27** (IC_{50} = 1.98 μM) was slightly less than that of adrimiacin (IC_{50} = 1.65 μM), but exhibited a 17-fold increase in comparison with OA (IC_{50} = 35.4 μM).

According to the assay results, succinylation of 3β-hydroxy in OA caused obvious decrease in potency against the growth of A549, MCF-7 and BGC-823 cells (**7** vs **1**). Interestingly, when the

free carboxylic acid group of succinyl group was amidated with taurine or at its α-position was added an L-hydroxy group, inhibitory activity recovered in the cell lines except for BGC-823 (**11** vs **7**, **17** vs **7**). As a result, taurine amide **11** and malic acid ester **17** were seven and 17 times more potent than their parent compound OA (**1**) in PC3 cell line, respectively, implying that the importance of H-bond donor group near C-3 position of OA. It seems that addition of H-donor group to this area could enhance the cytotoxicity in PC3, A549 and MCF-7 cell lines, which is identical with the observation in Hep G2 by Ma et al.[20] Generally, N-heterocycle-containing compounds **27**–**32** were less potent than **1**, **7**, **11** or **17**. For example, the potency of compound **31** sharply descended in all the four cancer cell lines. Lack of H-bond donor in A-ring area of these heterocycles might be one of the possible factors for this phenomenon. More general effects of N-heterocycle substitutes on activity are hard to conclude as they differed in particular cancer line. As for modification at C-17 position of oleanolic acid, replacement of the carboxy group by the carboxymethoxycarbonyl group leaded an overall downward trend of inhibition potency in all the four cancer cell lines (e.g., **10** vs **19**, **6** vs **16**, and **4** vs **14**). It has yet to determine whether anti-cancer activity prefers small size groups in this area.

In conclusion, 12 hydrophilic derivatives of oleanolic acid have been synthesized and biologically evaluated for cancer cell growth inhibition in PC3, MCF-7, A549, and BGC-823 cell lines. Several compounds have been found to have better or comparatable anti-cancer potency in comparison with adriamicin or oleanolic acid. Preliminary SAR analysis shows that introduction of H-bond donor substitution to the area near C-3 position of oleanolic acid may benefit the potency. Highly cytotoxic compounds **11** and **17** are more interesting than OA when drug-like properties are considered, and should be paid more attention since taurine and L-malic acid derivatives are widely used in development of water-soluble prodrugs.[25–27] Further research of hydrophilic derivatives of oleanolic acid as anti-cancer agents is ongoing in our laboratories.

Acknowledgements

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Supplementary data

Supplementary data (details of experimental procedures and characterisation data of synthesized compounds) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.01.129. These data include MOL files and InChiKeys of the most important compounds described in this article.

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