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Synthesis and cytotoxicity evaluation of oleanolic acid derivatives

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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.

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,

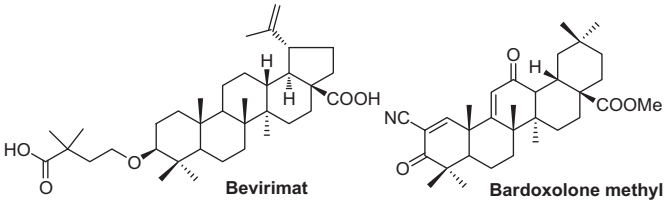

Figure 1. Structures of bevirimat and bardoxolone methyl.

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

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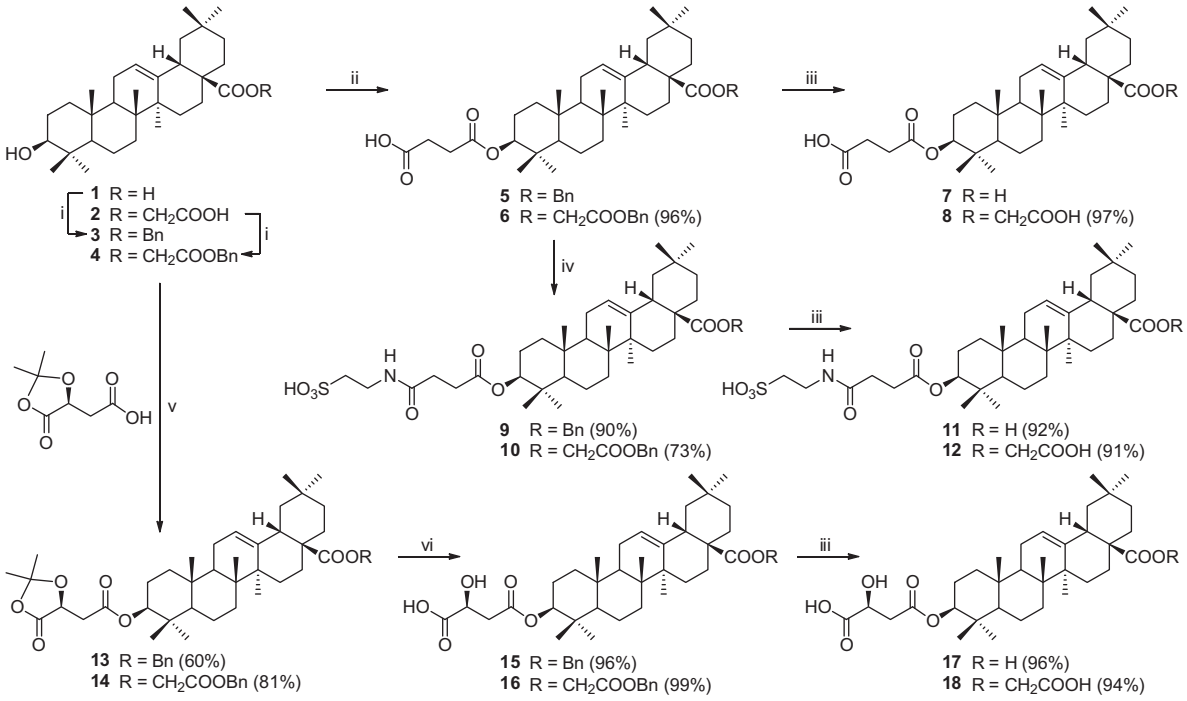
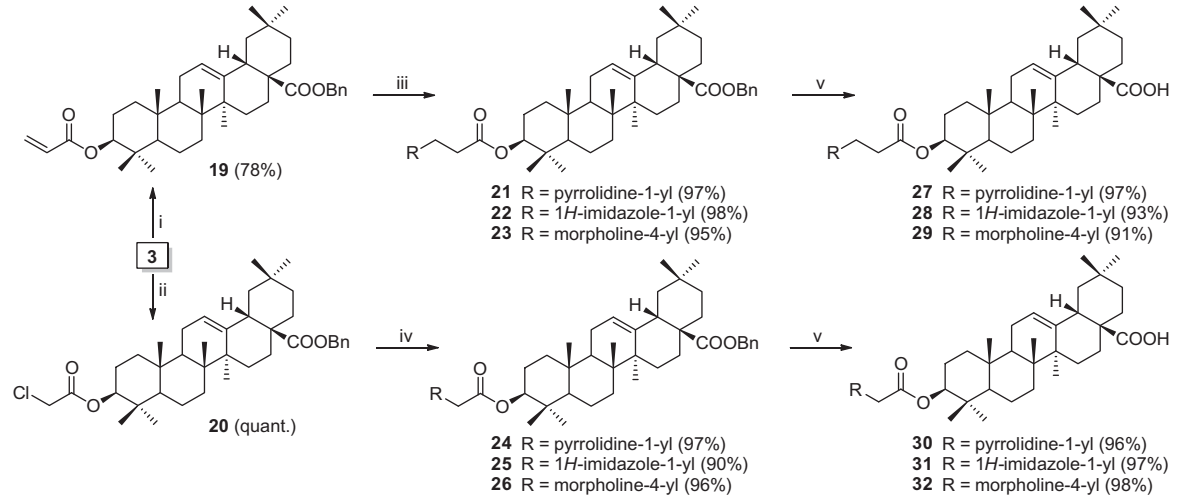


Scheme 1. Reagents and conditions: (i) BnCl/K_2CO_3/DMF; (ii) succinic anhydride/DMAP/pyridine; (iii) H_2/10% Pd–C/THF; (iv) *iso*-butyl chloroformate/Et_3N/THF, then taurine/Et_3N/CH_3CN; (v) DCC/DMAP/DCM; (vi) HAc/H_2O/THF.



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**,