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SYNTHESIS AND EVALUATION OF POTENTIAL COMPLEMENT INHIBITORY SEMISYNTHETIC ANALOGS OF OLEANOLIC ACID

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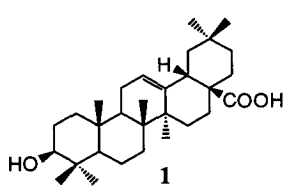
Abstract: A number of semisynthetic analogs of oleanolic acid have been synthesized and tested for their complement inhibitory, cytotoxic and apoptotic activities. Among these, compounds **10** and **17** exhibited complement inhibitory potency superior to oleanolic acid. Both have also shown a moderate improvement in in vitro therapeutic index (T.I.).

Activation of the complement system protects the body from invading organisms. However, unwanted and excessive activation contributes to the pathogenesis of various immune-mediated inflammatory diseases and hyperacute rejection of xenotransplants.[1–6] Thus, the complement system provides a target for therapeutic intervention in a variety of deleterious situations. Numerous compounds of natural or synthetic origin have been shown to inhibit the complement system.[7–10] No complement inhibitor, however, has been approved for clinical use by the U.S. FDA. Oleanolic acid (**1**), a triterpene natural product, inhibits the classical pathway of the complement system in vitro and complement-mediated inflammation in vivo.[11,12] The antitumor activity of oleanolic acid is well documented.[13] We also found oleanolic acid to have cytotoxic effects. A series of oleanolic acid semi-synthetic analogs have been synthesized and tested for their complement inhibitory activity to help delineate the structure-complement inhibitory relationships. These analogs involve modifications at the carboxyl, double bond and hydroxyl moieties of oleanolic acid. Because of the need to dissociate the cytotoxic property from the complement inhibitory activity, analogs have also been tested for cytotoxicity. Recognition of the structural similarity between oleanolic acid and the apoptosis inducer betulinic acid[14] (**2**) has prompted us to test oleanolic acid and its analogs for their ability to induce apoptosis.



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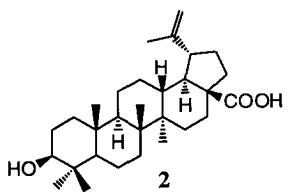

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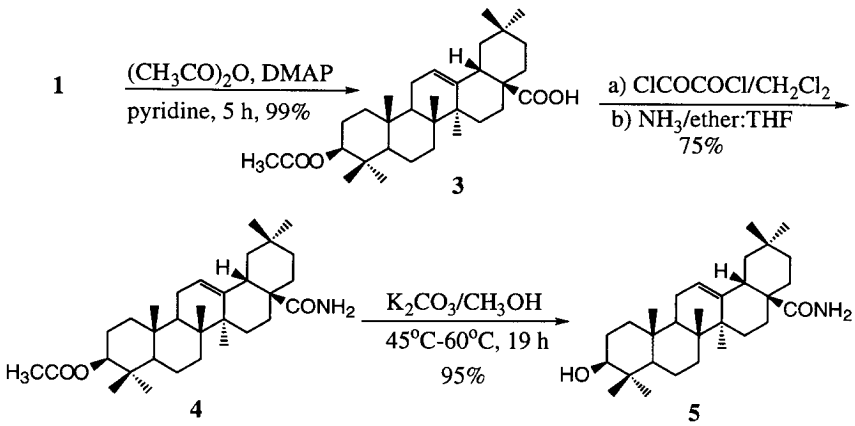
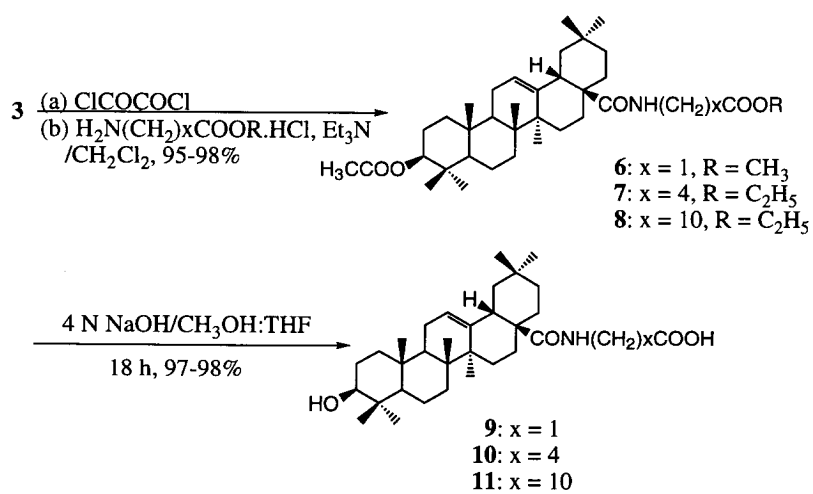
Many derivatives of oleanolic acid have been synthesized and tested for a variety of other biological activities.[15–17] Among these are the acyl derivatives **16–18** which have been reported by Lee et al. while this manuscript was under preparation.[18]

Scheme 1



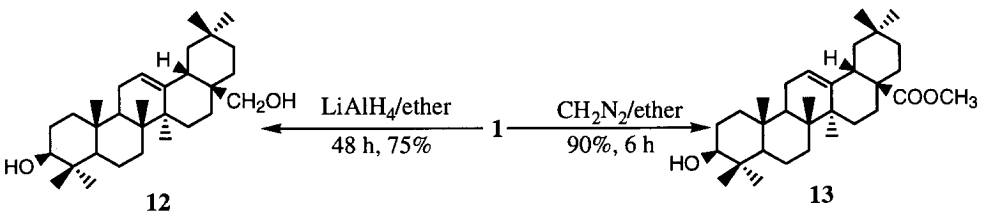
Compound **3** was prepared by stirring oleanolic acid with acetic anhydride in pyridine in the presence of 4-dimethylaminopyridine (DMAP). Conversion of 3-acetyloleanolic acid (**3**) to its corresponding acid chloride using oxalyl chloride followed by treatment with ammonia gave compound **4**. Deprotection of the hydroxyl group of **4** using potassium carbonate and methanol afforded oleanolic acid amide (**5**).[19]

Scheme 2

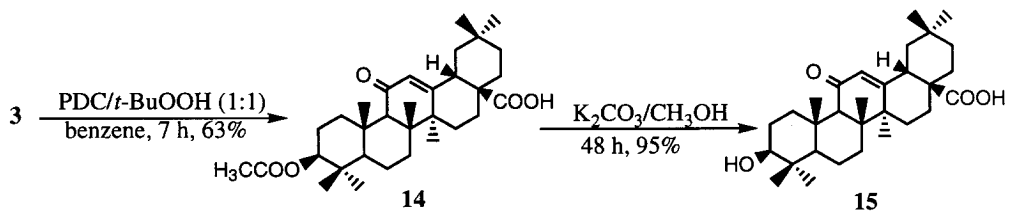


Treatment of the acid chloride of compound **3** with the corresponding ω-amino esters in the presence of triethylamine afforded amides **6–8**. Hydrolysis of the acetyl group and the methyl or ethyl esters of compounds **6–8** using aqueous sodium hydroxide yielded compounds **9–11**, respectively.[20]

Scheme 3

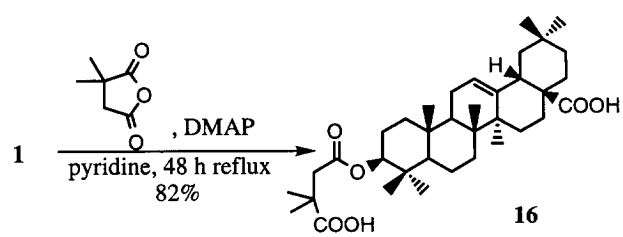


Scheme 4

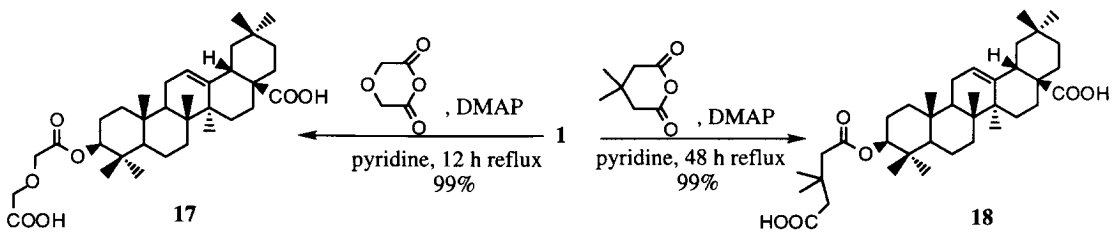


Reduction of oleanolic acid with lithium aluminum hydride afforded compound **12** while reaction with diazomethane yielded the methyl ester **13.** Compound **15** was synthesized by allylic oxidation of 3-acetyloleanolic acid (**3**) using pyridinium dichromate (PDC) and *tert*-butylhydroperoxide in 1:1 ratio[21] followed by alcoholysis of the acetyl group of compound **14** using methanol in the presence of potassium carbonate.

Scheme 5

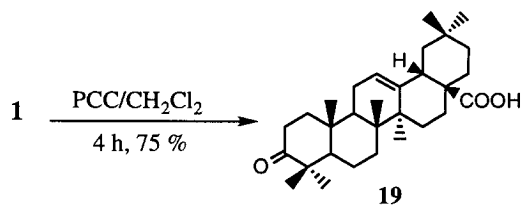


Scheme 6



The acyl derivatives, **16–18** were synthesized by refluxing oleanolic acid with their respective anhydrides in pyridine in the presence of DMAP.[22] It should be noted that treatment of oleanolic acid with 2,2-dimethylsuccinic anhydride gave exclusively compound **16** as shown by ^{1}H NMR spectrum. Oxidation of oleanolic acid using pyridinium chlorochromate (PCC) afforded compound **19**.

Scheme 7



All the synthesized compounds were tested in vitro for their inhibition of the classical pathway activation of human complement as previously described.[23] Compounds were assessed for cytotoxicity in a human malignant melanoma cell line, SK-MEL. Samples were incubated in a 96-well tissue culture-treated plate with 25,000 SK-MEL cells/well. Following a three day incubation, the number of remaining viable cells was assessed using the supravital dye, neutral red.[24]

DNA fragmentation was used to assess the presence of apoptosis. SK-MEL cells, 1×10^6/dish, were incubated with samples for 12 h. Following incubation, the cells were harvested and plasma membranes lysed. The lysate supernatant was digested with proteinase K in the presence of sodium dodecyl sulphate for 1 h at 50 °C. Isopropanol and sodium chloride were added to precipitate the DNA overnight at –20 °C. The pellet resulting from precipitation was suspended in buffer and RNA was digested for 2 h at 37 °C with RNAase A. Samples were run on a 1.5% agarose gel for 14 h at 70V and the gel was then stained with ethidium bromide.

The amide derivatives (compounds **9** and **10**), 11-oxooleanolic acid **15**, and the acyl derivatives (compounds **16–18**) have retained the complement inhibitory activity. Among these, compounds **10** and **17** show potency superior to oleanolic acid. Both have also shown a moderate improvement in in vitro T.I. and may serve as improved drug leads. Most of the derivatives tested have shown cytotoxic properties. Compounds **4** and **19** have cytotoxic potency similar to betulinic acid, but did not show complement inhibitory activity. The reported enhanced antitumor activity[17] of **19** compared to oleanolic acid might be related to its increased cytotoxicity. Although most of the compounds with cytotoxic activity have shown apoptotic activity, apoptosis was not consistently correlated with cytotoxicity.

Table 1. Classical complement inhibition, cytotoxicity, and apoptosis assay results for compounds **1–5** and **9–19**.

Compounds	Complement Inhibition IC_{50}, μM[a]	Cytotoxicity IC_{50}, μM[a]	T.I.[b]	Apoptotic Activity[c]
1	72.3 (±5.8)	112 (±8)	1.55	–
2	577(±103)	35 (±27)	0.06	+
3	na	na		–
4	na	70 (±14)		–
5	na	na		–
9	146.6 (±41.6)	115 (±69)	0.78	–
10	31.8 (±11.4)	127 (±34)	3.99	+
11	na	na		–
12	na	105 (±66)		–
13	na	103 (±61)		–
14	na	na		–
15	233.7 (±21.2)	131 (±32)	0.56	+
16	108.3 (±2.6)	77 (±37)	0.71	+
17	31.4 (±18.4)	93 (±35)	2.96	+
18	103.0 (±2.6)	86 (±59)	0.83	+
19	na	31 (±21)		+

[a]Values are means of three experiments, standard deviation is given in parentheses (na = not active).
[b]In vitro therapeutic index (IC_{50} cytotoxicity/IC_{50} complement inhibition)
[c]Apoptosis (+ = migration of DNA observed as a smear in the lane, – = no DNA migration observed).

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