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Enantioselective Synthesis and Complement Inhibitory Assay of A/B-Ring Partial Analogues of Oleanolic Acid

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Abstract—A series of oleanolic acid A/B-ring partial analogues was synthesized and tested for their complement inhibitory activity as well as cytotoxic properties. All target compounds and one intermediate exhibited moderate complement inhibitory potency. These compounds also showed cytotoxicity on malignant melanoma cell line, SK-MEL.

Complement is an essential component of the innate immune system that provides a first line defense and immune complex clearance in the blood stream.[1,2] Furthermore, complement plays a role in various functions of the adaptive immune response.[3,4] Yet overactivation of complement is implicated in various inflammatory diseases and xenotransplant rejection.[5–8] Complement inhibitors have been found to ameliorate these deleterious conditions, and a continued search for complement inhibitors has resulted in the identification of numerous natural and synthetic compounds.[9–11] However, no complement inhibitor has been approved for clinical use in the US due to the lack of potency and selectivity. The triterpene natural product, oleanolic acid (3β-hydroxy-olean-12-en-28-oic acid, **1**) inhibits the C3-convertase of the classical pathway in vitro.[12] Oleanolic acid also inhibits complement-mediated inflammation in animal models.[13] Earlier, we reported the synthesis and complement inhibitory activity of various semisynthetic analogues of oleanolic acid.[14] To further explore the regions of the molecule that confer complement inhibitory activity, we have designed and synthesized A/B-ring partial analogues of oleanolic acid. This dissection of the molecule also provides an easy access to a wider variety of analogues. The A/B-ring of oleanolic acid with its stereochemistry has been retained in the partial analogues while the carboxylic moiety has been tethered to the A/B-ring using a styrene (**2a**–**c**) or a benzyl spacer group (**3a**–**c**) (Fig. 1).

The optically pure compound **7** was synthesized using the method developed by Hagiwara and Uda.[15] Accordingly, condensation of 2-methyl-1,3-cyclohexanedione (**4**) with ethyl vinyl ketone in the presence of

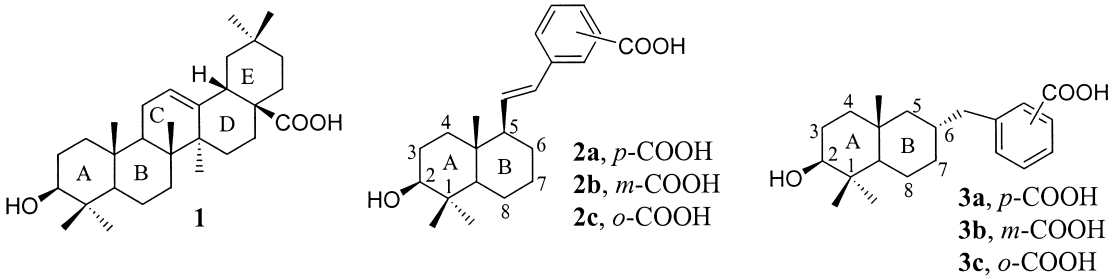

Figure 1. Oleanolic acid (**1**) and its partial analogues (**2a**–**c** and **3a**–**c**).

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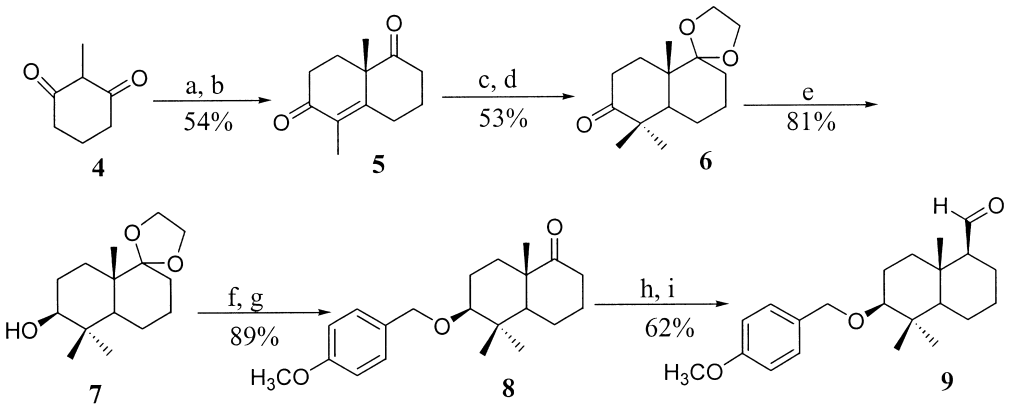
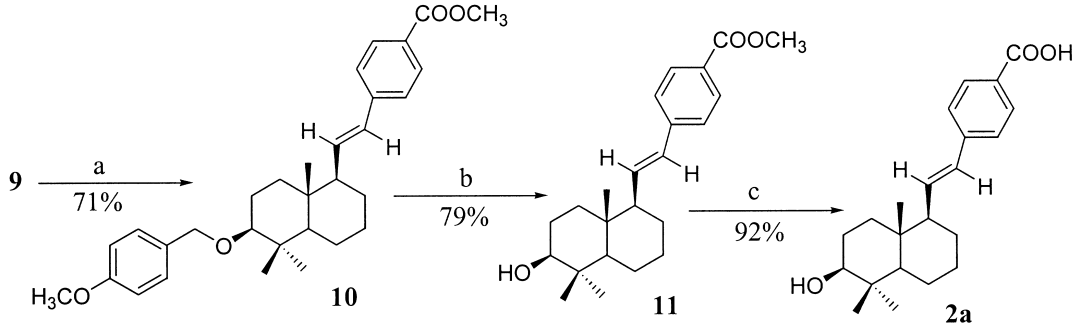


Scheme 1. Reagents and conditions: (a) ethyl vinyl ketone, Et_3N/THF, reflux; (b) L-Phe, *d*-CSA/DMF; (c) 2-ethyl-2-methyl-1,3-dioxolane, ethylene glycol, *d*-CSA, 50 °C; (d) (i) Li/liq NH_3; (ii) CH_3I; (e) $NaBH_4/C_2H_5OH$; (f) NaH, *p*-CH_3OPhCH_2Cl/DMF; (g) 1 N HCl/CH_3COOH/THF[1/2/3]; (h) NaH/DMSO, $CH_3OCH_2PPh_3Cl$; (i) HCl/THF.



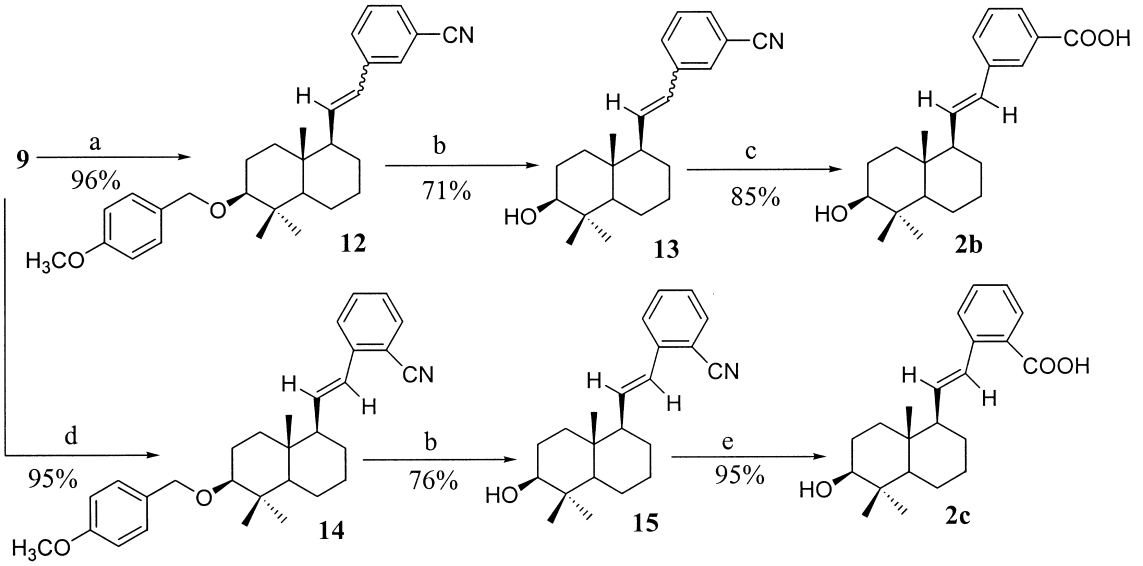
Scheme 2. Reagents and conditions: (a) NaH/DMSO, (*p*-carbomethoxybenzyl)triphenylphosphonium bromide; (b) $DDQ/CH_2Cl_2/H_2O$; (c) aq $NaOH/CH_3OH$/THF.

triethylamine under reflux followed by enantioselective cyclization of the resulting triketone using L-phenylalanine (L-Phe) as a chiral auxiliary afforded the *S*-enantiomer **5** as a major product. The pure *S*-enantiomer **5** obtained by crystallization was used as a starting material for the subsequent steps. Selective protection of the unconjugated keto group of **5** using 2-ethyl-2-methyl-1,3-dioxolane in the presence of *d*-camphorsulfonic acid (*d*-CSA) and treatment of the ketal with Li/liq NH_3 followed by iodomethane yielded the *trans*-decalin **6**. Reduction of the keto group of **6** using $NaBH_4$ gave the β-epimer **7** as the major product, which was separated from its α-epimer by flash chromatography to yield up to 81% of stereochemically pure **7**. Protection of the hydroxyl group of compound **7** using *p*-methoxybenzyl chloride and NaH and deprotection of the keto group using 1 N HCl and acetic acid in tetrahydrofuran resulted in the common intermediate **8**.[16] Wittig reaction of **8** with (methoxymethyl)triphenylphosphonium chloride using dimsylsodium as a base followed by hydrolysis of the resulting enol ether using hydrochloric acid in tetrahydrofuran (THF) afforded the β-aldehyde **9** as the major product.[17] Even though the α-epimer was formed as a minor product, column chromatographic separation gave a high yield of the pure epimer **9**.

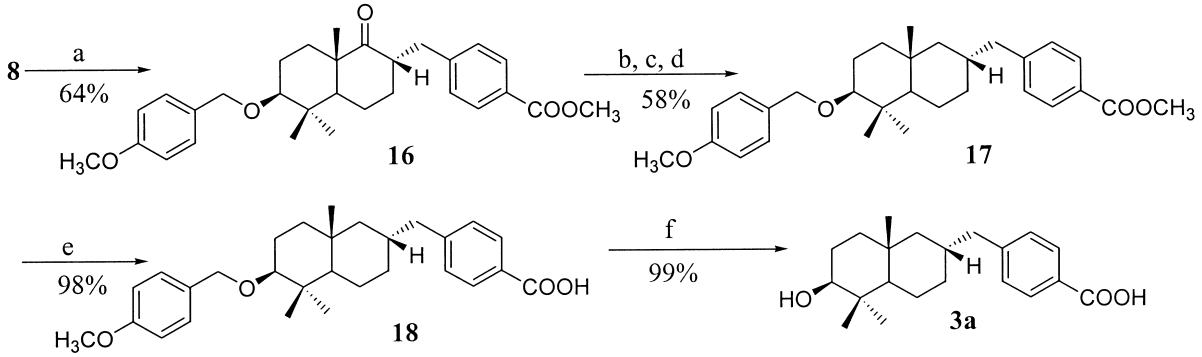
The stereochemistry of the aldehyde **9** was confirmed by 2-D NMR spectroscopic analysis. NOESY correlation between the angular methyl and the aldehyde protons clearly indicates the β-orientation of the formyl group. This stereochemical assignment was supported by molecular modeling studies.[18] The distance between the angular methyl and the formyl group hydrogens in the β-epimer **9** was calculated to be as close as 2.04 Å, while the minimum distance between these two hydrogens in the α-epimer was calculated to be 4.5 Å.

The synthesis of the target compounds **2a**–**c** began with the Wittig reaction of **9** with the respective triphenylphosphonium salts[19] using dimsylsodium as a base.[20] Thus, reaction of the aldehyde **9** with (*p*-carbomethoxybenzyl)triphenylphosphonium bromide gave the *trans* compound **10** as the major product, which was easily separable from its *cis*-isomer (minor product) by flash chromatography. Deprotection of the benzyl protecting group of **10** using 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ)[21] gave compound **11**, which on hydrolysis using NaOH yielded the compound **2a** as the final product.

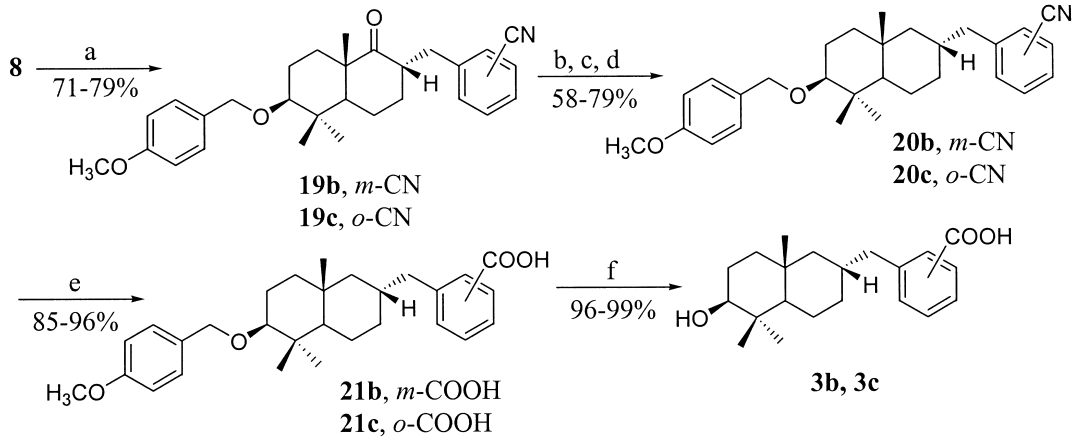
Reaction of **9** with (*m*-cyanobenzyl)triphenylphosphonium bromide yielded **12** as a *cis*–*trans* inseparable mixture (1:3 ratio, ^1H NMR). Therefore, the mixture was subjected to deprotection of the hydroxyl group with DDQ to give **13**. Hydrolysis of **13** using KOH in THF and methanol under reflux yielded the hydroxy acid (85%) along with the corresponding primary amide



Scheme 3. Reagents and conditions: (a) NaH/DMSO, (*m*-cyanobenzyl)triphenylphosphonium bromide; (b) DDQ/CH_2Cl_2/H_2O; (c) aq KOH/CH_3OH/THF, Δ; (d) NaH/DMSO, (*o*-cyanobenzyl)triphenylphosphonium bromide; (e) aq KOH/ethylene glycol, Δ.



Scheme 4. Reagents and conditions: (a) LDA, methyl *p*-(bromomethyl)benzoate/THF; (b) $NaBH_4$/C_2H_5OH; (c) NaH, CS_2, CH_3I/THF; (d) *n*-Bu_3SnH/toluene, Δ; (e) aq NaOH/CH_3OH/THF; (f) H_2/Pd–C.



Scheme 5. Reagents and conditions: (a) LDA, *m*-cyanobenzyl bromide or *o*-cyanobenzyl bromide/THF; (b) $NaBH_4$/C_2H_5OH; (c) NaH, CS_2, CH_3I/THF; (d) *n*-Bu_3SnH/toluene, Δ; (e) aq KOH/ethylene glycol, Δ; (f) H_2/Pd–C.

(15%). The pure *trans* product **2b** was obtained by crystallization from methanol. Wittig reaction of **9** with (*o*-cyanobenzyl)triphenylphosphonium bromide gave the *trans*-product **14** (>95%, ^1H NMR) as the major product. Cleavage of the *p*-methoxybenzyl group of **14** using DDQ afforded the alcohol **15**. Recrystallization afforded the pure *trans*-isomer, which was subjected to hydrolysis to give the final compound **2c**.

The synthesis of **3a–c** was initiated by alkylation of **8** using LDA and benzyl bromides in THF.[22] Therefore, alkylation of **8** with methyl *p*-(bromomethyl)benzoate afforded the α-epimer **16** as the major product. Recrystallization from hexane afforded the pure α-epimer **16**. Compound **16** was also obtained by stirring with K_2CO_3 in methanol probably due to the epimerization of the β-epimer (axial benzyl substituent) to the more stable α-epimer (equatorial benzyl substituent). The stereochemistry of **16** was assigned by 2-D NMR analysis in which the NOESY correlation between the angular methyl and the proton alpha to the carbonyl group was considered as a clear indication of the α-orientation of the benzyl substituent. The keto group was successfully converted to the corresponding methylene group by reducing the ketone to the corresponding alcohol using $NaBH_4$ and removing the resultant hydroxyl group using Barton deoxygenation protocol.[23] Hydrolysis of the ester group of **17** by stirring with aqueous NaOH in methanol and THF afforded **18**. Then the final product **3a** was obtained by hydrogenolysis of the *p*-methoxybenzyl group of **18** using Pd–C as a catalyst.

Alkylation of **8** with *m*-cyanobenzyl bromide or *o*-cyanobenzyl bromide afforded the α-epimer of compounds **19b** and **19c** as major products. The pure epimer (α-epimer) of each compound was obtained by stirring the mixture with K_2CO_3 in methanol. The relative stereochemistry was determined using the same method employed for the stereochemical assignment of compound **16**. Molecular modeling studies using the same program and methods described for **9** indicate that the distance between the angular methyl hydrogen and the hydrogen alpha to the carbonyl group is as close as 2.01 Å. The carbonyl group of **19b** and **19c** was reduced to the corresponding methylene group using the protocol described for the conversion of compound **16** to **17**. The target compounds **3b** and **3c** were obtained by hydrolysis of the cyano group of **20b** and **20c** using aq KOH in ethylene glycol under reflux, followed by hydrogenolysis of the *p*-methoxybenzyl group.

The compounds were bioassayed for the classical pathway complement inhibitory activity in vitro following the protocol described earlier.[24] The cytotoxic property of the compounds was assessed in a human malignant melanoma cell line, SK-MEL. Solution of the compounds were incubated with 25,000 SK-MEL cells/well for 72 h. The number of remaining viable cells was assessed using the supravital dye, neutral red.[25] Briefly, cells were washed with saline, incubated for 1.5 h with a 0.17% solution of neutral red in serum-free RPMI, and washed again to remove extracellular dye. Following solubilization with 0.04 N HCl in isopropanol, absorbance was read at 490 nM.

All the target compounds **2a–c** and **3a–c**, and the intermediate compound **21b** have shown moderate complement inhibitory potency (Table 1). The lack of complement inhibitory activity with compounds **11** and **15** shows the importance of the free carboxylic group for complement inhibition. The lack of complement inhibitory activity with compounds **18** and **21c** while compound **21b** retains the activity may indicate that the *meta* position of the carboxylic group is more favorable for complement inhibitory activity.

Table 1. Classical pathway complement inhibition and cytotoxicity assays of oleanolic acid and its partial analogues

Compd	Complement inhibition IC_{50} (μM[a])	Cytotoxicity IC_{50} (μM)	T.I.[b]
1	72.3 (±5.8)	112	1.55
11	na	na	—
2a	623 (±34)	380	0.6
15	na	155	—
2b	550 (±86)	269	0.5
2c	633 (±83)	380	0.6
18	na	200	—
3a	610 (±9)	378	0.6
21b	488 (±11)	180	0.4
21c	na	166	—
3b	601 (±51)	333	0.6
3c	616 (±66)	484	0.8

[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).

All the target compounds **2a–c** and **3a–c** as well as the intermediates **15**, **18**, **21b**, and **21c** showed cytotoxic activity. Cytotoxicity and complement inhibitory activity appear to be dissociated as indicated by compounds **15**, **18**, and **21c**. Although these partial analogues showed moderate complement inhibitory potency with low value of T.I., the flexible synthetic route developed should allow easy access to various analogues for further structure–activity relationship studies. It may also be possible to enhance the cytotoxicity to useful levels should the mechanism of cytotoxicity prove novel.

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