Pergamon

SCIENCE DIRECT

Bioorganic & Medicinal Chemistry Letters 13 (2003) 3137–3140

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Synthesis and Cytotoxic Activity of A-ring Modified Betulinic Acid Derivatives

Young-Jae You,[†] Yong Kim, Nguyen-Hai Nam and Byung-Zun Ahn*

College of Pharmacy, Chungnam National University, Taejon 305-764, South Korea

Received 5 July 2003; accepted 11 July 2003

Abstract—New A-ring modified betulinic acid derivatives having small steric hindrance were prepared and tested for cytotoxic activity on 3 cancer cell lines: 10 compounds showed stronger cytotoxic activity than betulinic acid. Especially, the compounds bearing 1-ene-3-oxo with electron-withdrawing groups at C2 showed strong cytotoxicity.

Introduction

Lupane derivatives are prevalent in natural sources and have various biological activities. Betulinic acid, one of well known lupanes, showed anti-HIV[1,2] and anti-inflammatory activity.[3] Moreover, its selective cytotoxic activity towards human melanoma cells (SK-MEL-2) gained more interest from researchers.[4,5] The cytotoxic activity of betulinic acid was reported to come mainly from the induction of apoptosis.[6,7] Our group has studied structure–activity relationships between structures of lupanes and cytotoxicities against various tumor cell lines:[8] the carboxylic acid of C-28 seemed to be essential for activity which was consistent with other groups' results[5] and introduction of bulky group at C-3 and C-28 interfered with cytotoxicity.[9] On the other hand, in the course of developing nitric oxide inhibitors from oleanolic acid and ursolic acid, modification of ring A of triterpenoids, which took relatively small steric hindrance, resulted in increased biological activity.[10–13] In this article we report the synthesis of betulinic acid derivatives with modification of ring A and their cytotoxic activities.

Synthesis of A-ring Modified Derivatives

To introduce the 2-cyano-3-oxo-1-en moiety on A-ring, Scheme 1 was used. Compound **6** was synthesized from **2** using sodium methoxide and ethyl formate by 83% yield which was confirmed by a singlet peak of methylene at 8.59 ppm in the ^{1}H NMR. After the isoxazole ring formation (**10**, 80%), compound **14** was synthesized by ring opening with sodium methoxide (97%) followed by oxidation with DDQ to give **17** (60%). The singlet peak correspondent to H at C1 appeared at 7.80 ppm in the ^{1}H NMR spectrum. Compounds **18** and **19** were synthesized by the same procedures used for **17**. Unexpectedly, when R_3 was carboxylic acid (**9**), the reaction did not give the anticipated product in the ring opening step.

In Scheme 2, the double bond [20(29)] of betulonic acid (**1**) was saturated and carboxylic acid (C28) was protected to a methyl ester. Using phenylselenyl chloride and *m*CPBA, **20**, which has a 3-oxo-1-ene moiety, was formed by 63% yield. Via epoxy derivative, **22**, 2-methoxy-1-ene and 2-chloro-1-ene moieties (**23**, **25**) were synthesized with NaOMe and hydrochloric acid, respectively. Demethylation of methyl esters was performed with LiH in DMF in fairly good yields (about 60%).

Compound **26** was synthesized from **27** using phenylselenyl chloride and 30% H_2O_2 in 65% yield which was confirmed by two singlet peaks at 9.98 and 7.87 ppm in the ^{1}H NMR representing aldehyde and C1 proton, respectively (Scheme 3).

Biological Results and Discussion

Structures and their biological results were summarized in Table 1. As mentioned in the introduction, we focused on the structural effects of A-ring modification without steric burden on cytotoxic activity. Along with

*Corresponding author at present address: SB Pharmaceutical Company, Bonggok-li 533-2, Banpo-myun, Gongju-shi 314-923, South Korea. Tel.: +41-857-3686; fax: +41-857-7854; e-mail: ahnbj@sbpp.co.kr
[†]Present address. Department of Chemistry, University at Buffalo, The State University of New York, Buffalo, NY 14260, USA.

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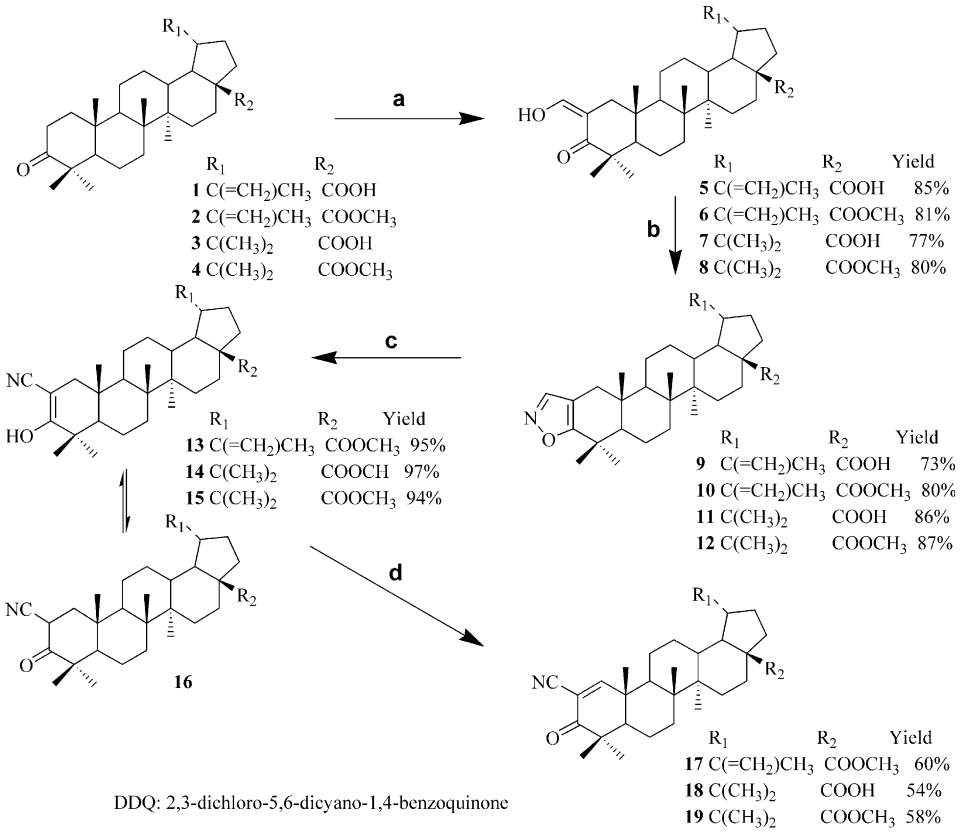
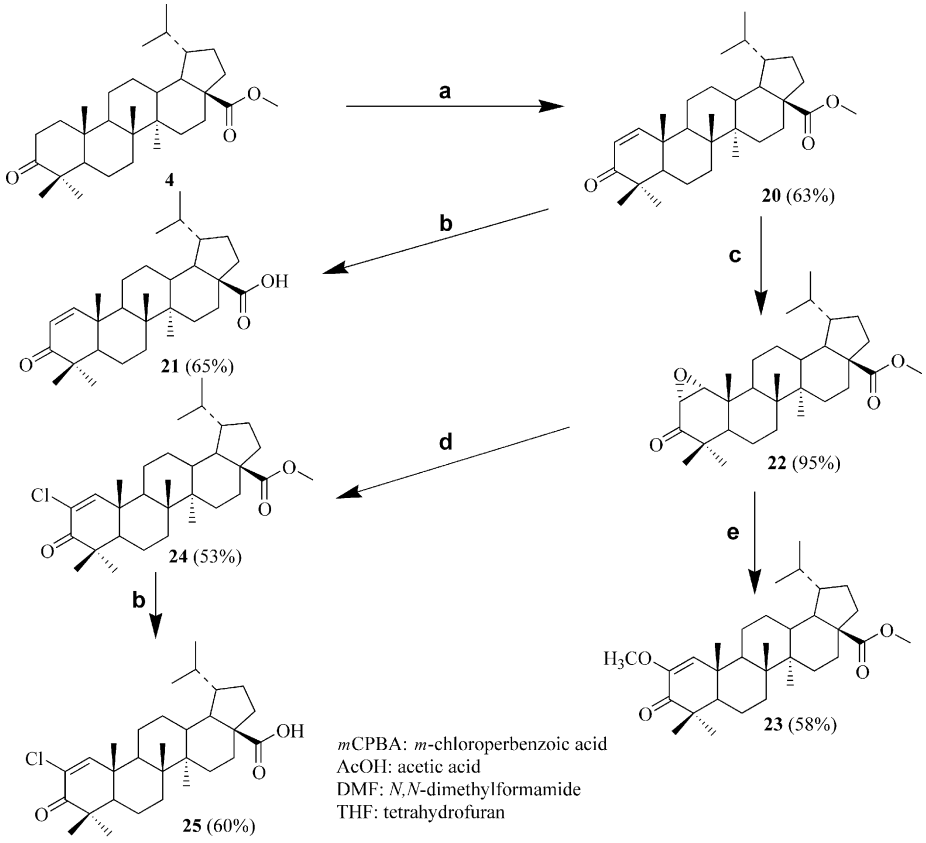
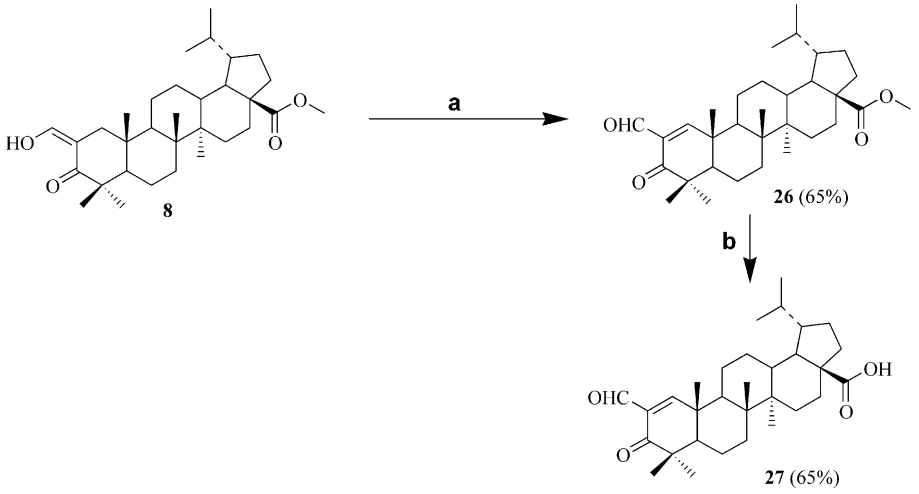
doi:10.1016/S0960-894X(03)00724-8



Scheme 1. Reagents and conditions: (a) HCO_2Et, NaOMe; (b) NH_2OH, aq EtOH; (c) NaOMe, Et_2O, MeOH; (d) DDQ, Benzene.



Scheme 2. Reagents and conditions: (a) PhSeCl, EtOAc then *m*CPBA, pyridine, EtOAc; (b) LiI, DMF; (c) 30% H_2O_2, aq NaOH, THF; (d) HCl, AcOH, $CHCl_3$; (e) NaOH, MeOH.

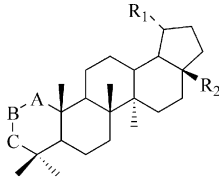


Scheme 3. Reagents and conditions: (a) PhSeCl, pyridine, CH_2Cl_2 then 30% H_2O_2; (b) LiI, DMF.

this, we could observe the effects of double bond (R_1) and carboxilic acid (R_3) moiety. Electron-withdrawing groups at C2 of 3-oxo-1-en moiety strengthened the cytotoxicity. Among A-ring modified derivatives with free acid at C28, 2-cyano-, 2-chloro-, or 2-formyl-3-oxo-1-en derivatives, **18**, **25** and **27** were strongly cytotoxic. Especially in case of **25**, the strength increased by 59 times more than the lead compound, betulinic acid (**BA**). For most derivatives the free carboxilic acid moiety in C28 seemed to be critical in cytotoxic activity. Importance of the carboxylic acid moiety was already suggested for other derivatives.[2] Compound **5**, 2-hydroxymethylene-3-oxo derivative, showed specific cytotoxicity on A-549 cell and **9** was the most cytotoxic towards A-549 cells, while their methyl esters, **6** and **10**, were not cytotoxic (ED_{50}, $>30\mu g/mL$). The same phenomenon was observed for compound pairs of **7** versus **8**, **11** versus **12** and **25** versus **24**.

The double bond at R_1 was not critical: the saturated forms, **7** and **11**, were more cytotoxic than their unsaturated forms, **5** and **9**. More interestingly, methyl esters of 2-cyano- or 2-formyl-3-oxo-1-en derivatives, **17**, **18** and **26**, were strongly cytotoxic, implying that 2-formyl- or 2-cyano-3-oxo-1-en moiety itself plays an important role in cytotoxic action. The common feature of those

Table 1. Cytotoxic activities of A-ring modified lupane derivatives



Compd	R_1	-A-B-C-	R_2	M2[a]	A-549	B16[b]
5	Isopropenyl	2-Hydroxymethylene-3-oxo–	Carboxyl	>30[c]	2.76[c]	>30[c]
6	Isopropenyl	2-Hydroxymethylene-3-oxo–	Methylcarboxyl	>30	>30	>30
9	Isopropenyl	[2,3-d]Isoxazolo–	Carboxyl	16.6	1.54	3.11
10	Isopropenyl	[2,3-d]Isoxazolo–	Methylcarboxyl	>30	>30	>30
17	Isopropenyl	2-Cyano-3-oxo-1-en–	Methylcarboxyl	2.12	4.55	3.57
7	Isopropyl	2-Hydroxymethylene-3-oxo–	Carboxyl	3.76	2.20	1.66
8	Isopropyl	2-Hydroxymethylene-3-oxo–	Methylcarboxyl	>30	>30	>30
11	Isopropyl	[2,3-d]Isoxazolo–	Carboxyl	1.15	2.70	1.57
12	Isopropyl	[2,3-d]Isoxazolo–	Methylcarboxyl	>30	>30	>30
18	Isopropyl	2-Cyano-3-oxo-1-en–	Carboxyl	0.81	1.32	1.43
19	Isopropyl	2-Cyano-3-oxo-1-en–	Methylcarboxyl	1.25	13.0	0.80
20	Isopropyl	3-Oxo-1-en–	Methylcarboxyl	>30	>30	>30
21	Isopropyl	3-Oxo-1-en–	Carboxyl	2.29	2.89	1.75
22	Isopropyl	1α,2α-Epoxy-3-oxo–	Methylcarboxyl	>30	>30	>30
23	Isopropyl	2-Methoxy-3-oxo-1-en–	Methylcarboxyl	>30	>30	>30
24	Isopropyl	2-Chloro-3-oxo-1-en–	Methylcarboxyl	>30	>30	>30
25	Isopropyl	2-Chloro-3-oxo-1-en–	Carboxyl	0.13	1.01	1.55
26	Isopropyl	2-Formyl-3-oxo-1-en–	Methylcarboxyl	0.73	0.31	0.29
27	Isopropyl	2-Formyl-3-oxo-1-en–	Carboxyl	0.26	0.17	0.30
BA				7.62	7.70	4.98

[a]M2: SK-MEL-2.
[b]B16: B16-F10.
[c]ED_{50} (μg/mL).

functional groups was the strong electro-negativity making an attack of bionucleophiles more favorable.

In conclusion, A-ring modification with the least steric burden, along with the introduction of electron-withdrawing group at C2 of the 1-ene-3-oxo moiety has yielded several strong cytotoxic derivatives of betulinic acid. It was found that the presense of the double bond at 20(29) was not essenatial for the cytotoxic activity, while the carboxylic acid at C28 was very important.

Acknowledgements

This work was supported by SB Pharmaceutical Company, Gongju-shi, Korea.

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