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Synthesis and activity of oleanolic acid derivatives, a novel class of inhibitors of osteoclast formation

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Abstract—Two series of oleanolic acid derivatives were synthesized and their inhibitory activity on the formation of osteoclast-like multinucleated cells (OCLs) induced by 1α,25-dihydroxy vitamin D_3 was evaluated in a co-culture assay system. The structure–activity relationships, together with electronic structure based on the frontier molecular orbitals, for example, HOMO and LUMO, related to different amino acid substituents were studied. Derivatives with proline or phenylalanine showed a tendency to enhance the inhibitory activity.

Osteoporosis is a disease characterized by low bone mineral density and structural deterioration of bone tissue, which leads to bone fragility and increased susceptibility to fractures.[1] The disease is widely recognized as a major public health problem, particularly among postmenopausal women. The cause of the disease is thought to stem from an imbalance of the bone remodeling process with bone resorption exceeding bone formation. Since potentiated bone resorption by osteoclasts, the primary bone-resorption cells, is one of the major causes of osteoporosis, osteoclasts are one of the targets of chemotherapy for osteoporosis. Inhibitors of osteoclasts may therefore present useful agents to prevent the excessive bone resorption associated with osteoporosis.[2]

In searching of biologically active substances from topical traditional herbal drugs, we have screened a number of crude drug extracts for their inhibitory activity on bone resorption that stimulated by parathyroid hormone (PTH) in a bone tissue culture system.[3] Among those extracts, a methanol extract from the root of *Achyranthes bidentata* Blume (Amaranthaceae) showed the most potent activity. In vivo studies showed that the butanol soluble fraction of the methanol extract prevented the bone loss characteristic of osteoporosis in OVX rats effectively.[4] Further chemical isolation and structure elucidation associated with in vitro assay indicated that the active components are oleanolic acid type of triterpenoids. We also discovered that oleanolic acid, the genin of these triterpenoids, have inhibitory effect of bone resorption stimulated by PTH.

Oleanolic acid (**1**), a natural product, has been reported to have numerous pharmacological activities including anti-inflammatory,[5] anti-cancer,[6] anti-HIV,[7] and hepato-protective effects.[8] Many derivatives of oleanolic acid have been synthesized for a variety of other biological activities.[9–12] It was also reported that amino acid conjugates of natural product could produce effective antitumor agents[13] and modification of ring C of oleanolic acid could increase the inhibitory potency of nitric oxide production.[11] These reports, along with our own preliminary screening results of oleanolic acids as potential anti-osteoporosis lead compounds prompted us to embark on an investigation to modify the structure to improve the inhibitory activity on osteoclast formation.

Keywords: Oleanolic acid derivatives; Inhibitor; Osteoclast formation; Frontier molecular orbitals.
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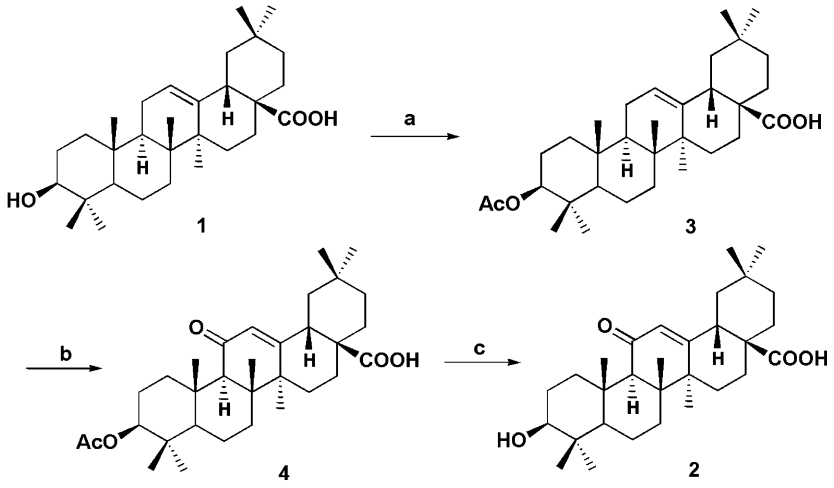
To explore the effects of the amino acid substituents and other functional groups on anti-osteoporosis agents, we converted C_{11}-methylene to a carbonyl group and C_{17}-carboxyl to an amide using several natural amino acids. In this paper, we describe the synthesis, inhibitory activity of osteoclast formation and structure–activity relationships for these compounds.

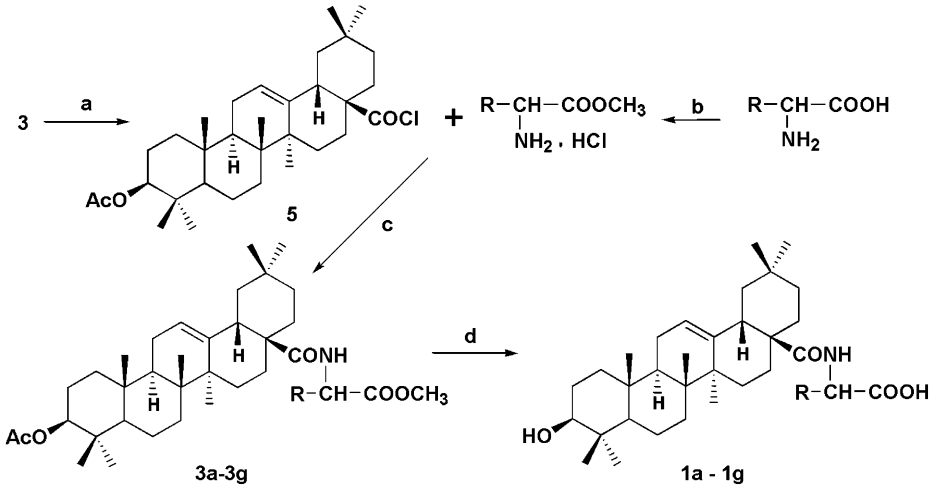
The preparation of compound **2** was outlined in Scheme 1. 3-Acetyloleanolic acid (**3**) was synthesized by stirring oleanolic acid with acetic anhydride in dry pyridine in the presence of 4-dimethylaminopyridine (DMAP). On oxidation of compound **3** with excess of *tert*-butyl chromate in dry carbon tetrachloride at 65 °C overnight afforded 3-acetyl-11-oxooleanolic acid (**4**) in 53% yield.[14] Deprotection of the hydroxyl group of **4** using potassium carbonate in methanol at 45–60 °C for 19 h gave 11-oxooleanolic acid (**2**).[15]

A general synthesis for compounds **1a**–**g** were conducted according to the following procedures (Scheme 2). Compound **5** was prepared by stirring 3-acetyloleanolic acid (**3**) with oxalyl chloride in dry CH_2Cl_2. Treatment of compound **5** with corresponding amino acid methyl esters[16] in the presence of triethylamine in CH_2Cl_2 afforded amides **3a**–**g**. Hydrolysis of the acetyl group and the methyl esters of compounds **3a**–**g** using aqueous sodium hydroxide yielded compounds **1a**–**g**, respectively. Compounds **2a**–**g** were synthesized in the same protocol as **1a**–**g** from 3-acetyl-11-oxooleanolic acid (**4**) (Fig. 1).

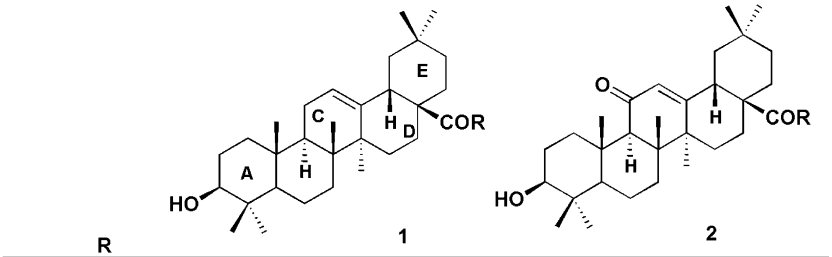
Two series of compounds **1**, **1a**–**g** and **2**, **2a**–**g** (Fig. 1) were tested for their inhibitory activity on 1α,25-dihydroxy vitamin D_3 [1α,25$(OH)_2D_3$] induced TRAP-positive osteoclast-like multinucleated cells (OCLs) formation in a co-culture assay system with mouse bone marrow cells and osteoblast-like cells reported by Takahashi et al.[17] Elcatonin, a clinically available anti-osteoporosis drug was used as a positive control.[18] As shown in Table 1, all of the tested compounds except for **1b**, **2a**, **2b**, **2d** retained inhibitory effects on OCLs formation. Introducing proline and phenylalanine at C_{28}-position of lead compound (**1**) displayed an increase tendency in inhibitory effects compared with **1**. How-



Scheme 1. Reagents and conditions: (a) Ac_2O/DMAP/pyridine, rt, 99%; (b) (*tert*-BuO)$_2CrO_2$/CCl_4, 53%; (c) K_2CO_3/MeOH, 95%.



Scheme 2. Reagents and conditions: (a) ClCOCOCl/CH_2Cl_2; (b) $SOCl_2$/MeOH, 83–86%; (c) Et_3N/CH_2Cl_2, 90–93%; (d) 4 M NaOH, MeOH/THF, 95–98%.



R		1	2
$HNCH_2COOH$	(Gly)	1a	2a
$HNCHCOOH$ CH_3	(Ala)	1b	2b
$HNCHCOOH$ $CH(CH_3)_2$	(Val)	1c	2c
$HNCHCOOH$ $H_3CH_2C{\cdot}CHCH_3$	(Ile)	1d	2d
$HNCHCOOH$ $CH_2CH(CH_3)_2$	(Leu)	1e	2e
$HNCHCOOH$ CH_2Ph	(Phe)	1f	2f
$N-CHCOOH$	(Pro)	1g	2g

Figure 1.

Table 1. Effects of tested compounds on $1\alpha,25(OH)_2D_3$ induced TRAP(+)-OCLs formation

Compd	OCLs (%)	Compd	OCLs (%)
Control	$100.0 \pm 16.2^{\#}$		
Elcatonin	$29.0 \pm 9.1^{**}$	Normal	20.0 ± 10.0
1	$27.6 \pm 8.5^{**}$	**2**	$38.5 \pm 3.8^{**}$
1a	$61.5 \pm 12.0^{*}$	**2a**	83.3 ± 11.7
1b	87.8 ± 2.9	**2b**	76.3 ± 20.0
1c	$41.3 \pm 9.5^{*}$	**2c**	$59.0 \pm 12.8^{*}$
1d	$53.5 \pm 7.9^{*}$	**2d**	62.0 ± 15.1
1e	$51.2 \pm 10.7^{*}$	**2e**	$69.5 \pm 8.3^{*}$
1f	$16.3 \pm 7.9^{**}$	**2f**	$36.5 \pm 14.4^{**}$
1g	$21.0 \pm 4.7^{**}$	**2g**	$40.3 \pm 4.9^{**}$

Control: cultured with $1\alpha,25(OH)_2D_3$ (10^{-8} M). Elcatonin: cultured with $1\alpha,25(OH)_2D_3$ (10^{-8} M) and elcatonin (2 U/mL). Normal: cultured without any additions. Samples: cultured with $1\alpha,25(OH)_2D_3$ (10^{-8} M) and each compound (20 μM). Each value was expressed as mean ± SD, $n = 4$. The data of control group was pegged as 100% (OLC number was 104), while other data were calculated relative to it. Data were analyzed by student's t test. Significant differences in TRAP(+)-OCLs compared with control group, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{\#}p < 0.05$ versus normal group.

ever, other amino acid derivatives showed less activity. The alanine derivatives of both **1** and **2** were inactive. The skeleton modification (C_{11}-methylene of to a carbonyl group) of the lead compound (**1**) led to a decrease in activity compared with **1**. Furthermore, all of the derivatives of **2** were less active in comparison with the parent compound and the corresponding derivatives of **1**. These results suggested that the C_{11}-methylene group of lead compound is important for inhibitory potency.

Recently, a theoretical approach has been used to correlate electronic indices to the biological activity of a class of carcinogenic polycyclic aromatic hydrocarbons (PAHs) and derived a simple rule to predict bioactivity of PAHs.[19] This methodology was based on the energy separation values between frontier molecular orbitals and in their relative contribution to the local density of electronic states over specific molecular regions. In order to further explore their structure–activity relationships, a preliminary work on semi-empirical and ab initio calculations, which concerns the locations and the relative energies of the frontier molecular orbitals, namely HOMO (the highest occupied molecular orbital) and LUMO (the lowest unoccupied molecular orbital) on all of the synthesized molecules, was taken at the semi-empirical parametric method 3 (PM3) and HF/3-21* levels, respectively. The final RMS gradient less than 0.01 kcal/mol was achieved for all fully optimized molecules. The results indicated that the geometrical structures of derivatives of **1** were similar to those of corresponding ones of **2**, however, surprisedly, a big difference on the locations and the relative energies of HOMO and LUMO (E_{HOMO} and E_{LUMO}) was observed. For the compound **1** and its derivatives, HOMO mainly located on ring C and partially on ring D, and LUMO on the double bond and tended to move to the amino acid substituent if used. On the contrary, for the compound **2** and derivatives HOMO mainly located on the double bond and amino acid substituent, and LUMO on ring C. A graphitic comparison of HOMO and LUMO of representative molecules, **1f** and **2f**, was given in Figure 2. For the E_{HOMO} and E_{LUMO}, each derivative of **1** possessed higher level than each corresponding one of **2** (Table 2). In addition, in **1** and its derivatives, **1**, **1f**, and **1g** with more potent activity showed higher E_{HOMO}, which accounts for the electron donor ability, the same tendency was observed in **2** and its derivatives except for **2c** and **2d**, which will

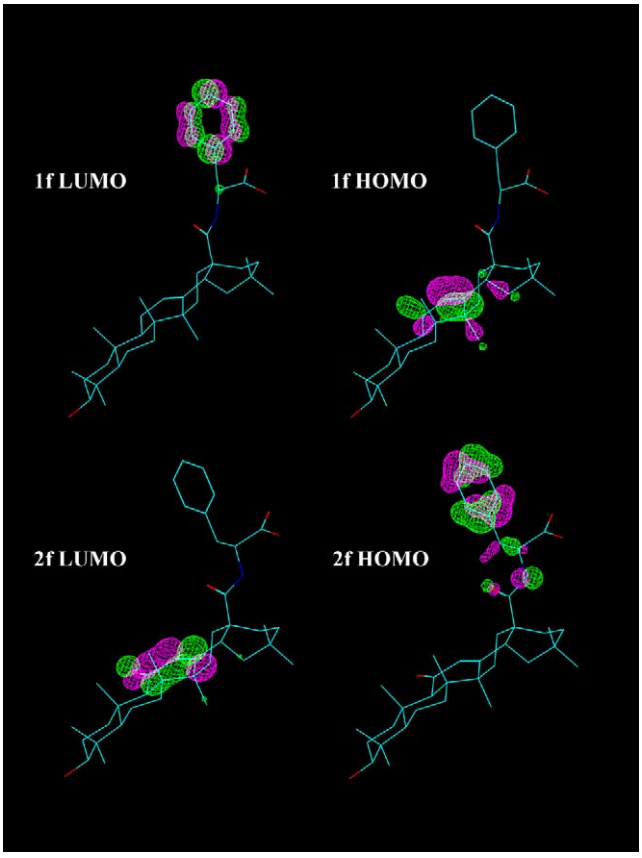


Figure 2. Comparison of the frontier molecular orbitals HOMO and LUMO of compounds **1f** and **2f**.

Table 2. Energies (eV) of the frontier molecular orbitals for the OA derivatives

Compd	E_{HOMO}	E_{LUMO}	Compd	E_{HOMO}	E_{LUMO}
1	−9.4197	1.0450	**2**	−10.1327	−0.0741
1a	−9.5186	0.5616	**2a**	−10.1610	−0.1515
1b	−9.4879	0.6895	**2b**	−10.1628	−0.1517
1c	−9.4979	0.7840	**2c**	−10.0674	−0.1585
1d	−9.5010	0.8179	**2d**	−10.0711	−0.1623
1e	−9.4940	0.7725	**2e**	−10.1427	−0.1551
1f	−9.4375	0.1894	**2f**	−9.6960	−0.1108
1g	−9.3953	0.8632	**2g**	−9.6819	−0.0594

The calculations were carried out at semi-empirical PM3 level.

be investigated further. These results suggested that conversion of the C_{11}-methylene to a carbonyl group strongly impacted the energies and locations of HOMO and LUMO, and the compound with high E_{HOMO} might have the potent activity. It is worth to indicate that a lot of efforts remained in order to reach a simple rule to predict the activity. Hopefully, further detailed analyses could clarify the relationship between the electronic structure and activity, create electronic indices including the optimal locations and the energies of HOMO and LUMO, environmental aspects, such as hydrophobicity, and give a guide to synthesis of the more potent inhibitors.

In summary, oleanolic acid derivatives **1f** and **1g** showed a high inhibitory activity on formation of OCLs. Compound **1f** was the most potent with 16.3% inhibition of OCL formation relative to the control (100%). Proline and phenylalanine substituents of oleanolic acid showed a tendency to enhance the activity, other amino acids that used in the experiments had no benefit on improvement of the activity. Removal of the hydrogen atoms on C_{11} gave a big impact on the electronic structure and could be a major cause of decrease of anti-OCLs formation activity. The locations and the relative energies of HOMO and LUMO might closely relate to the activity. Further syntheses of derivatives, studies on bioactivities and analyses of the frontier molecular orbitals HOMO and LUMO are in progress and will be reported in due course.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2005.01.061.

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