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Pentacyclic triterpenes. Part 3: Synthesis and biological evaluation of oleanolic acid derivatives as novel inhibitors of glycogen phosphorylase

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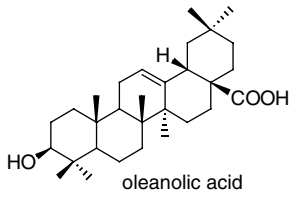
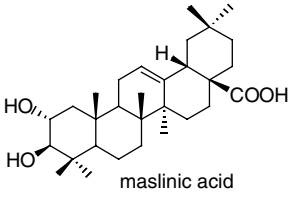
Abstract—Oleanolic acid and its synthetic derivatives have been identified as novel inhibitors of glycogen phosphorylase. Within this series of compounds, **4** (IC_{50} = 3.3 μM) is the most potent GPa inhibitor. Preliminary structure–activity relationships of the oleanolic acid derivatives are discussed.

Oleanolic acid (OA), which has been in active clinical use as an anti-hepatitis drug in China for over 20 years, possesses some attractive biological activities including protection of the liver against toxic injury,[1] anti-inflammation,[2] anti-HIV,[3] and antitumor.[4] Liu et al.[5] reported that OA exhibited hypoglycemic effect in alloxan-induced hyperglycemic rats, meanwhile, significant accumulation of hepatic glycogen in rats was observed in OA treatment groups compared with the control groups, indicating a possible inhibition of hepatic glycogenolysis. The action mechanism of OA's effect on hepatic glycogen metabolism, however, remains unknown.



oleanolic acid



maslinic acid

Very recently, we first reported that maslinic acid, a natural pentacyclic triterpene abundant in olive fruit, represented a new class of inhibitors of glycogen phosphorylase (GP).[6] GP is the enzyme responsible for glycogen breakdown to produce glucose and related metabolites for energy supply.[7] Due to its key role in modulation of glycogen metabolism, pharmacological inhibition of GP has been regarded as a promising therapeutic approach for treating diseases caused by abnormalities in glycogen metabolism.[8] Several structural classes of GP inhibitors have been developed,[8a,8b,9] and a couple of GP inhibitors have been in clinical or pre-clinical trials, for example, PSN-357 is an oral GP inhibitor which is currently in phase II clinical trial for treatment of type 2 diabetes.[10]

Based on our previous studies, we further examined other members of pentacyclic triterpene family with respect to their inhibitory effects on GP. The enzyme assay results showed that like maslinic acid (IC_{50} = 28 μM), OA was also a naturally occurring GP inhibitor (IC_{50} = 14 μM). Encouraged by this in vitro result, we tested hypoglycemic activity of OA in adrenaline-induced diabetic mice which had hyperglycemia due to increased hepatic glycogenolysis. OA was administered orally at 100 mg/kg/day for 7 days, and on the last day, after adrenaline was iv administered, blood glucose levels of OA treatment groups and the control

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Table 1. Effect of oleanolic acid (OA) on fasted plasma glucose of hyperglycemic mice induced by adrenaline ($n = 10$)

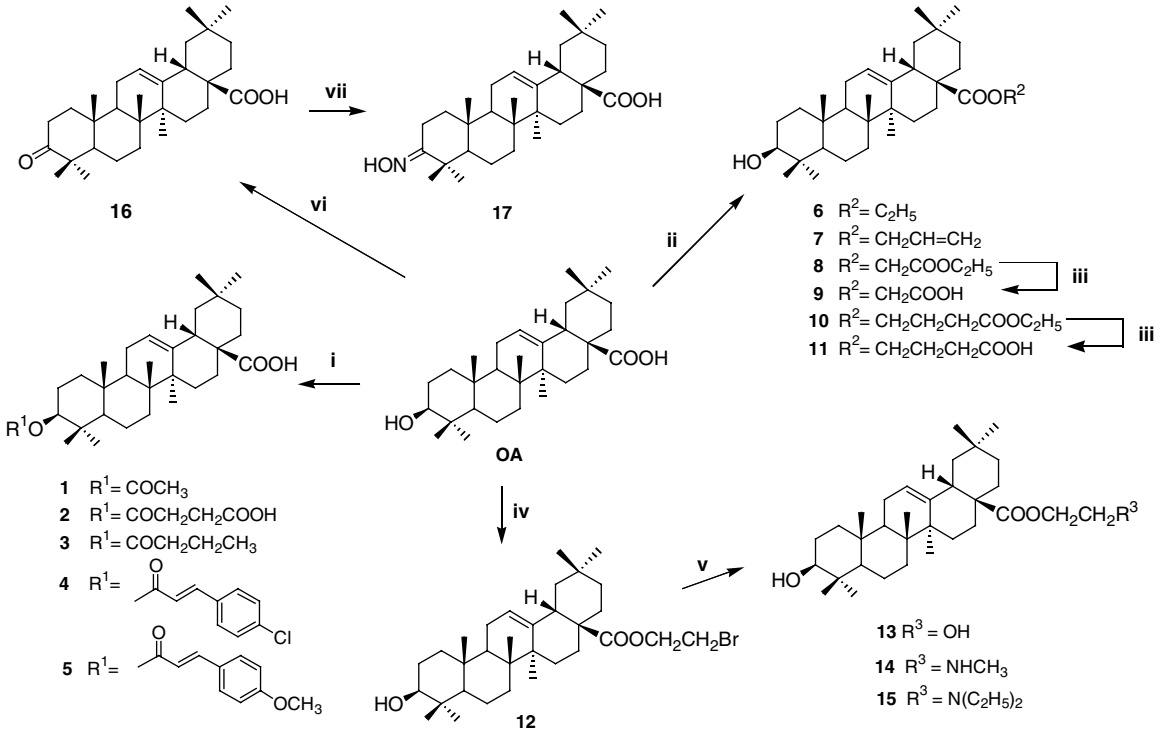
Compound	Dose (mg/kg)	Fasted plasma glucose (OD)		
		0 h	1 h	2 h
Vehicle		0.138 ± 0.028	0.212 ± 0.032	0.149 ± 0.037
OA	100	0.114 ± 0.020	0.153 ± 0.040	0.063 ± 0.025

groups were measured. Not surprisingly, the in vivo test result (Table 1) showed that OA effectively inhibited the increase of fasted plasma glucose of diabetic mice induced by adrenaline.

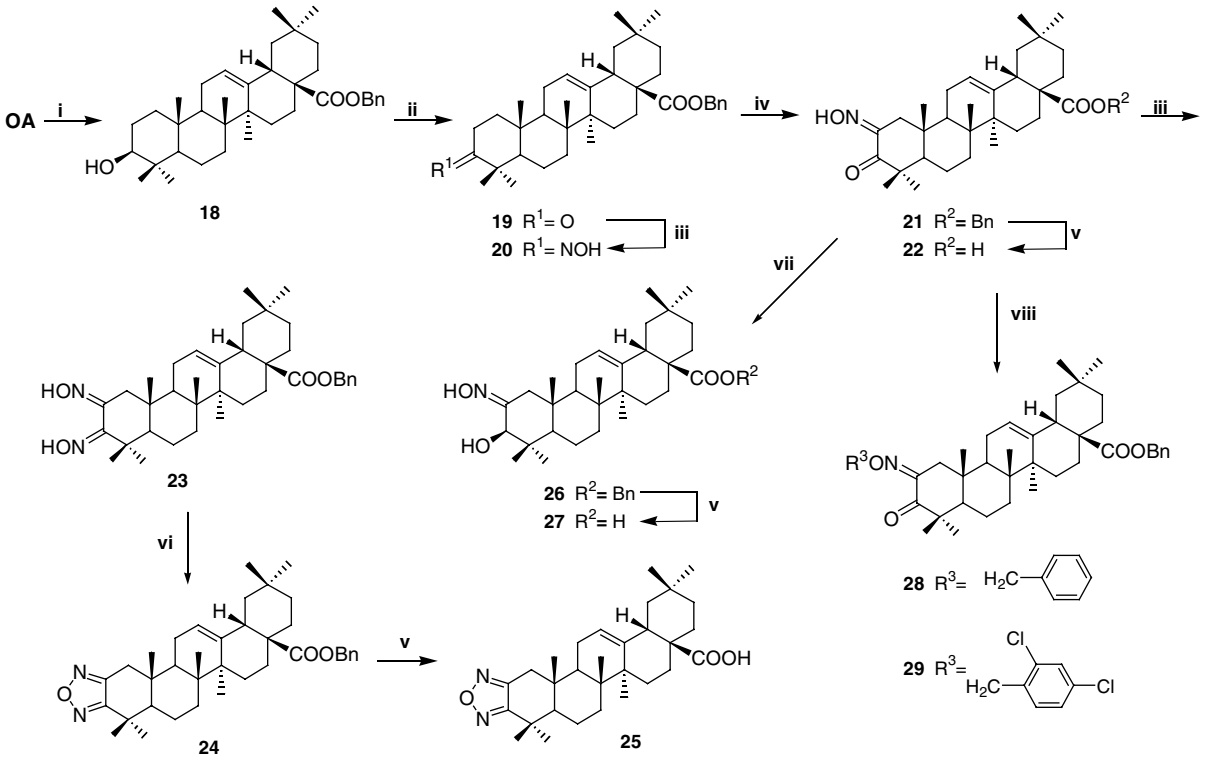
The finding that OA is a natural GP inhibitor is of particular importance due to the following points: (1) it has been disclosed herein that OA may lower blood glucose, at least in part, through inhibiting GP and therefore reducing hepatic glucose production. (2) OA, as a safe nonprescription drug for the treatment of hepatitis, may find its new clinical uses in treating diabetes characterized by fasting hyperglycemia and other diseases caused by disorders in glycogen metabolism. (3) OA, which is widely distributed in plant kingdom, is very cheap and easily available in large bulk, and therefore, lead modification based on OA would be in a good position for further drug development.

With OA as a lead compound, we sought to identify a series of OA derivatives as novel GP inhibitors. Initial synthetic efforts were focused on structural modifications at C-3 and C-28 positions. The synthetic routes are outlined in Scheme 1. Treatment of OA with anhydride/pyridine or RCOOH/DCC/DMAP or RCOCl/Et_3N afforded 3-*O*-acyl OA derivatives **1**–**5** in 40–91% yields. C-28 esters **6**–**8** and **10** were prepared in high yields (85–93%) by esterification of OA in the presence of K_2CO_3 in DMF with bromoethane, allyl bromide, ethyl bromoacetate, and ethyl 4-bromobutyrate, respectively. Hydrolysis of **8** and **10** with 1 M NaOH in THF/MeOH gave the corresponding carboxylic acids **9** and **11** in quantitative yields, respectively. Reaction of OA with 1,2-dibromoethane in the presence of K_2CO_3 in DMF furnished bromide compound **12** (86%), which was further converted to hydroxyl ethyl ester **13** (78%) by heating of **12** with aqueous ethanol in the presence of Et_3N. Treatment of **12** with aqueous methylamine solution in the presence of K_2CO_3 in acetone at room temperature afforded amine **14** in 75% yield. Reaction of **12** with diethylamine in the presence of K_2CO_3 in acetone at reflux temperature gave amine **15** in 72% yield. Jones oxidation of OA afforded oleanonic acid **16** (87%). Reaction of **16** with hydroxylamine hydrochloride in pyridine at 80 °C afforded oxime **17** (82%).

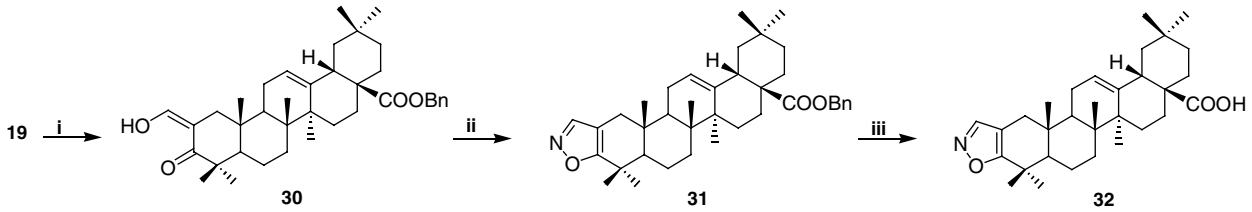
Next, we attempted to probe the effect of structural modification at A-ring of OA on GP inhibitory activity. In this regard, some oxime, oxadiazole, and isoxazole derivatives of OA were synthesized as depicted in Schemes 2 and 3. Esterification of OA with benzyl chloride followed by an oxidation reaction with PCC afforded ketone **19** in high yields.[6] Treatment of **19** with hydroxylamine hydrochloride in pyridine gave oxime



Scheme 1. Reagents and conditions: (i) anhydride/Pyr, 80 °C or RCOOH/DCC/DMAP, THF, rt, or RCOCl/Et_3N, rt (40–91%); (ii) R^2Br, K_2CO_3, DMF, rt (85–93%); (iii) 1 M NaOH, THF/MeOH, rt (quant.); (iv) $BrCH_2CH_2Br$, K_2CO_3, DMF, 40 °C (86%); (v) for **13**: Et_3N, aqueous ethanol, reflux (78%); for **14**: aqueous methylamine solution, K_2CO_3, acetone, rt (75%); for **15**: diethylamine, K_2CO_3, acetone, reflux, (72%); (vi) CrO_3/H_2SO_4/acetone/H_2O, 0 °C (87%); (vii) $HONH_3Cl$, pyridine, 80 °C (82%).



Scheme 2. Reagents and conditions: (i) BnCl, K_2CO_3, DMF, 60 °C (95%); (ii) PCC, CH_2Cl_2, rt (89%); (iii) $HONH_3Cl$, Pyr, 80 °C (80–87%); (iv) $NaNO_2$, H_2SO_4, $CH_3OH/THF/H_2O$, rt (58%); (v) H_2, Pd/C, THF, rt; (vi) NaOH, 1,2-ethanediol, 200 °C, 30 min (82%); (vii) $NaBH_4$, THF, 0 °C (78%); (viii) benzyl chloride or 2,4-dichlorobenzyl chloride, K_2CO_3, DMF, 40 °C (50–55%).



Scheme 3. Reagents and conditions: (i) HCOOEt, CH_3ONa, CH_2Cl_2, rt (80%); (ii) $HONH_3Cl$, aq EtOH, 80 °C, 2 h (95%); (iii) H_2, Pd/C, EtOAc, rt, 2 h (12%).

20 (87%). Introduction of the oximino group to the C-2 position of **19** was carried out by reaction of **19** with sodium nitrite under acidic conditions to give α-oximinoketone **21** (58%). Treatment of **21** with hydroxylamine hydrochloride in pyridine at 80 °C afforded dioxime **23** (80%). Cyclization of **23** was performed by heating **23** with NaOH in 1,2-ethanediol at 200 °C to give oxadiazole **24** (82%). Hydrogenolysis of **24** over Pd/C in THF at room temperature gave oxadiazole carboxylic acid **25**[11] in quantitative yield. Reduction of **21** with $NaBH_4$ in THF at 0 °C afforded α-oximinoalcohol **26** in 78% yield. Alkylation of **21** with benzyl chloride and 2,4-dichlorobenzyl chloride in the presence of potassium carbonate in DMF gave oxime ethers **28** and **29** in moderate yields (50–55%), respectively. Hydrogenolysis of **21** and **26** over Pd/C in THF at room temperature gave the corresponding carboxylic acids **22** and **27** in quantitative yield, respectively. Formylation of **19** with ethyl formate in the presence of sodium methoxide in CH_2Cl_2 gave compound **30** (80%). Treatment of **30** with hydroxylamine hydrochloride in aqueous EtOH at reflux temperature afforded isoxazole **31** (95%). Hydrogenolysis of **31** over Pd/C in EtOAc at room temperature was a complex reaction to yield the desired isoxazole carboxylic acid **32** in poor yield (12%).

The synthesized OA derivatives were evaluated in the enzyme inhibition assay against rabbit muscle glycogen phosphorylase a which shared considerable sequence similarity with human liver GPa. As described previously,[12] the activity of rabbit muscle GPa was measured through detecting the release of phosphate from glucose-1-phosphate in the direction of glycogen synthesis. The assay results (Table 2) showed that many synthesized OA derivatives exhibited moderate inhibitory activity against rabbit muscle GPa.

Unlike maslinic acid derivatives which had a clear preference for hydrophobic groups at C-28 for GP inhibition,[6b] the OA derivatives showed some uncertainty in

Table 2. Inhibition of rabbit muscle GPa by compounds **1**–**32**

Compound	RMGPa IC_{50}[a] (μM)
1	NI[b]
2	34.3
3	NI
4	3.3
5	16.9
6	NI
7	NI
8	NI
9	62.6
10	76.7
11	NI
12	NI
13	NI
14	53.2
15	NI
16	17.9
17	225
18	461
19	66.6
20	20.8
21	46.8
22	26.1
23	22.3
24	1002
25	11.2
26	179.1
27	28.2
28	61.3
29	8.0
30	6.3
31	19.6
32	12.7
OA	14
Caffeine	114

[a] Values are means of three experiments.
[b] NI, no inhibition.

terms of inhibitory potency correlated with the hydrophobic properties of C-28 substituents (e.g., **8** vs **9** and **10** vs **11**). Introduction of aminoethyl esters at C-28 in order to improve aqueous solubility resulted in decreases in potency (e.g., **14** and **15**). In most cases, C-28 carboxylic acids were more potent than their benzyl esters (e.g., **21** vs **22**, **24** vs **25**, **26** vs **27**, and **31** vs **32**), indicating a preference for the free carboxylic acid group at C-28.

Assay result for 3-*O*-acyl series showed that cinnamic analogues **4** (IC_{50} = 3.3 μM) and **5** (IC_{50} = 16.9 μM) were potent GPa inhibitors, while acetate **1** and butyrate **3** were inactive. Compound **4** was 3-fold more potent than OA, indicating that A-ring of OA might be a good target for further lead optimization. 2-Oximino series of compounds (**21**–**23** and **26**–**28**) were less potent than OA, except for **29** (IC_{50} = 8 μM). Oxadiazole **25** (IC_{50} = 11.2 μM) and isoxazole **32** (IC_{50} = 12.7 μM) exhibited similar enzyme inhibitory potency with OA, indicating that A-ring fused heterocyclic analogues might deserve some attention for further inhibitor design. Conversion of OA to oleanonic acid (**16**, IC_{50} = 17.9 μM) did not result in a significant change in potency.

In summary, we have identified oleanolic acid and its synthetic derivatives as novel GPa inhibitors. As a possible result of this finding, oleanolic acid, a nonprescription anti-hepatitis drug, may find its new clinical uses in treating fasting hyperglycemia and other diseases caused by abnormalities in glycogen metabolism.[13] Lead optimization based on oleanolic acid resulted in a series of triterpene class of GP inhibitors, among which, **4** was the most potent GPa inhibitor (IC_{50} = 3.3 μM). Further research and drug development on pentacyclic triterpene compounds as promising GPa inhibitors are ongoing in our laboratory and the results will be reported in due course.

Acknowledgments

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11. Analytical data for compound **4**: ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.78 (3H, s), 0.91 (6H, s), 0.93 (6H, s), 0.97 (3H, s), 1.15 (3H, s), 2.81–2.85 (1H, m), 4.64 (1H, t, J = 8.0 Hz), 5.29 (1H, br s), 6.41 (1H, d, J = 16.0 Hz), 7.35 (2H, d, J = 8.5 Hz), 7.46 (2H, d, J = 8.6 Hz), 7.61 (1H, d, J = 16.0 Hz); ^{13}CNMR ($CDCl_3$, 300 MHz): 15.4, 16.8, 17.2, 18.2, 23.0, 23.4, 23.6, 25.9, 27.7, 28.1, 30.7, 32.6, 32.8, 33.1, 33.8, 37.0, 38.0, 38.1, 39.3, 41.0, 41.6, 45.9, 46.5, 47.6, 55.4, 81.2, 119.5, 122.6, 129.1, 129.2, 133.1, 136.1, 142.9, 143.6, 166.6, 182.8; ESIMS: 619 $[M-H]^-$. Analytical data for compound **25**: IR (KBr, cm^{-1}) 2947, 2869, 1693, 1460, 1386, 1002, 881; ^{1}HNMR ($CDCl_3$, 300 MHz): δ 0.83, 0.84, 0.92, 0.94, 1.18, 1.31, 1.41 (each 3H, s), 2.17 (1H, d, J = 16.3 Hz, H_α-1), 3.10 (1H, d, J = 16.2 Hz, H_β-1), 2.87 (1H, dd, J = 3.9, 13.6 Hz, H-18), 5.36 (1H, t, J = 3.5 Hz, H-12); ^{13}CNMR ($CDCl_3$, 300 MHz): 15.4, 16.6, 19.1, 22.9, 23.3, 23.5, 24.7, 25.7, 27.7, 30.7, 31.3, 31.8, 32.4, 33.0, 33.2, 33.8, 35.3, 38.5, 39.4, 41.1, 41.9, 45.8, 45.9, 46.6, 53.1, 122.1, 143.7, 150.5,159.7,183.3; ESIMS: 481 $[M+H]^+$, 503 $[M+Na]^+$.
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