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Pentacyclic triterpenes. Part 2: Synthesis and biological evaluation of maslinic acid derivatives as glycogen phosphorylase inhibitors

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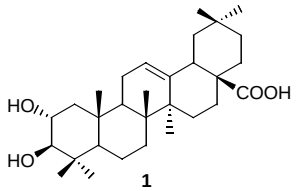
Abstract—The synthesis of a series of maslinic acid derivatives is described and their effect on rabbit muscle glycogen phosphorylase a evaluated. Within this series of compounds, **15** (IC_{50} = 7 μM) is the most potent GPa inhibitor. SAR of the maslinic acid derivatives are discussed.

Energy metabolism, which mainly involves fat metabolism, glucose metabolism, and protein metabolism, is one of the basic characteristics of biological processes. Multiple abnormalities in energy metabolism, especially abnormalities in glucose metabolism, result in a variety of severe diseases. As part of our project aimed at pharmacological interference with glucose metabolism, we have interest in developing glycogen phosphorylase inhibitors as therapeutic agents. Glycogen phosphorylase (GP) is the enzyme responsible for glycogen breakdown to produce glucose and related metabolites for energy supply.[1] Due to its key role of GP in modulation of glycogen metabolism, pharmacological inhibition of GP has been regarded as an effective therapeutic approach for treating diseases caused by abnormalities in glycogen metabolism, such as type 2 diabetes,[2] myocardial ischemia,[3] and tumors.[4] Several structural classes of GP inhibitors have been reported, whose binding sites identified in GP include the catalytic site, the purine inhibitor site (also known as I-site), the allosteric site, the glycogen storage site, a novel allosteric inhibitor site, and the newly discovered benzimidazole-binding site.[5]

Maslinic acid (**1**), which is an abundant constituent of olive fruit, has recently drawn much interest due to its anti-tumor,[6] anti-HIV,[7] antioxidation,[8] and antiobesity activities.[9] More recently, we have first reported that **1** and related triterpenes represent a new class of GP inhibitors.[10] This discovery afforded pentacyclic triterpenes as novel lead compounds in searching for potent and low-toxic GP inhibitors as therapeutic agents for type 2 diabetes and other diseases. In our previous studies, **1** exhibited moderate inhibition against both rabbit muscle GP and rat liver GP.[10] Moreover, **1** effectively inhibited the increase of fasted plasma glucose of diabetic mice induced by adrenaline.[10] In an effort to clarify SAR of the triterpene class of GP inhibitors, the present study has mainly focused on structural modifications at C-28, C-2, and C-3 positions of **1**. Herein, we describe the synthesis, biological evaluation, and SAR of maslinic acid derivatives as a new class of GP inhibitors.



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Keywords: Glycogen phosphorylase; Inhibitor; Maslinic acid; Derivatives; Synthesis; SAR.
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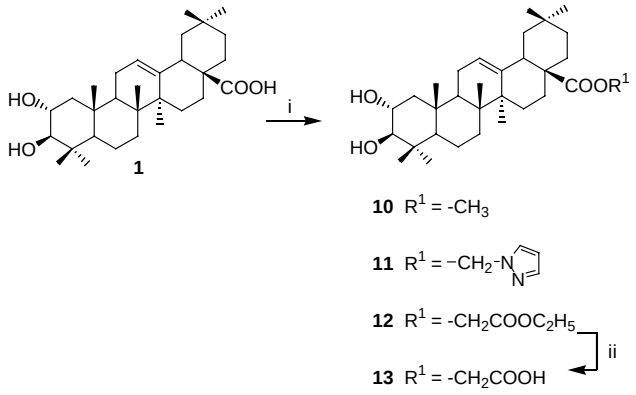
The syntheses of maslinic acid derivatives are summarized in Schemes 1–4. Maslinic acid (**1**) was synthesized based on a hydroboration-oxidation reaction as described previously.[10] On the other hand, Takayama and co-workers reported the preparation of corosolic acid via ketone-hydroxylation strategy,[11] and based on this methodology, we employed modified procedures to carry out the preparation of **1** and related compounds (Scheme 1). It was found that protection of the carboxyl groups as benzyl esters was very convenient and necessary, since process manipulation on the triterpene acids was very tedious and low-yielding. Thus, esterification of **2** with benzyl chloride followed by an oxidation reaction with PCC afforded ketone **4** in high yields.[10] Stereoselective hydroxylation of **4** with mCPBA catalyzed by H_2SO_4 in MeOH-CH_2Cl_2 at 0 °C gave 2α-hydroxyl ketone **5** in 80% yield. Reduction of **5** with $NaBH_4$ in THF at 0 °C gave 2α,3β-diol **6** (77%) as the major product, together with 2α,3α-diol **7** (18%) as a minor product. The relative stereochemistry at C-2 and C-3 of **6** and **7** was determined by ^1H NMR coupling data ($J = 9.5$ Hz in response to *trans*-coupling between H-3α and H-2β in **6**; $J = 2.6$ Hz in response to *cis*-coupling between H-3β and H-2β in **7**). Hydrogenolysis of **6** or **7** over palladium/carbon in THF furnished maslinic acid (**1**) or 3-*epi*-maslinic acid (**8**) in quantitative yield, respectively. It should be mentioned that **5** was unstable at basic conditions, for example, treatment of **5** with KOH in MeOH-DMF at room temperature gave α,β-unsaturated ketone **9** in almost quantitative yield.

Treatment of **1** with iodomethane in the presence of potassium carbonate in DMF at room temperature afforded methyl ester **10** in 95% (Scheme 2). In a similar fashion, maslinic acid esters **11** and **12** were obtained in good yields upon treating **1** with *N*-chloromethylpyrazole[12] and ethyl bromoacetate, respectively. Ester **12** was further converted to **13** in quantitative yield by

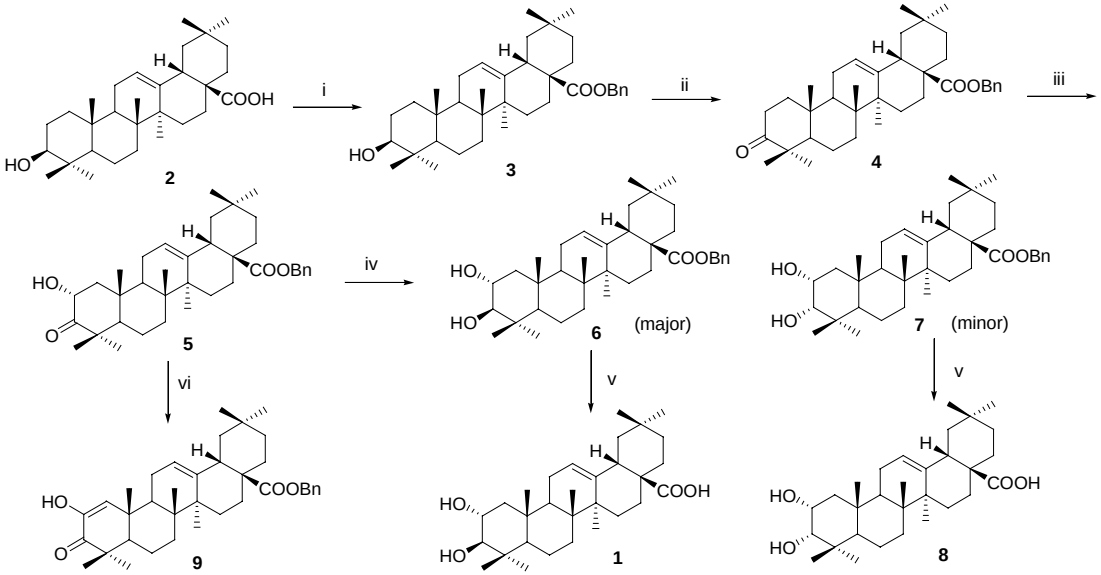


Scheme 2. Reagents: (i) R^1X, K_2CO_3, DMF; (ii) THF-4 N NaOH. ($R^1X = CH_3I$, *N*-chloromethylpyrazole or ethyl bromoacetate, respectively).

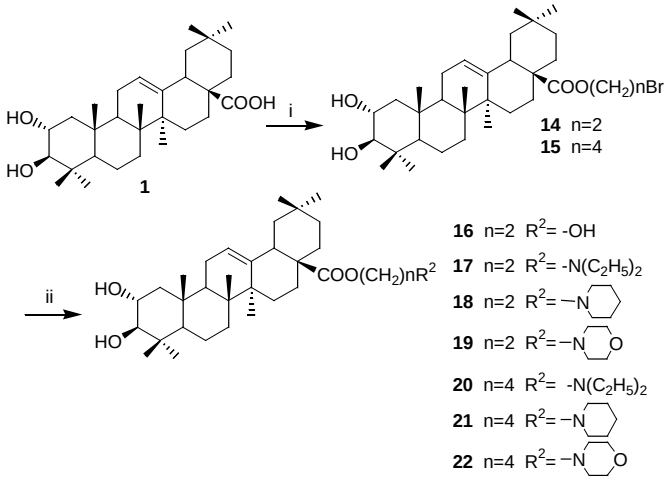
hydrolysis of **12** with 4 N NaOH aqueous solution in THF without effecting the ester bond of C-28.

Reaction of **1** with 1,2-dibromoethane or 1,4-dibromobutane in the presence of potassium carbonate in DMF at room temperature gave compounds **14** and **15** in high yields, respectively (Scheme 3). Treatment of **14** in refluxing aqueous ethanol in the presence of Et_3N afforded**16** in 78% yield. Nitrogen-containing derivatives **17**–**22** were synthesized through reactions of **14** or **15** with corresponding amines. For example, a mixture of **14**, diethylamine, and potassium carbonate in DMF was stirred overnight at room temperature to give **17** in 82% yield. In the same way, compounds **18**–**22** were prepared in moderate to good yields.[13]

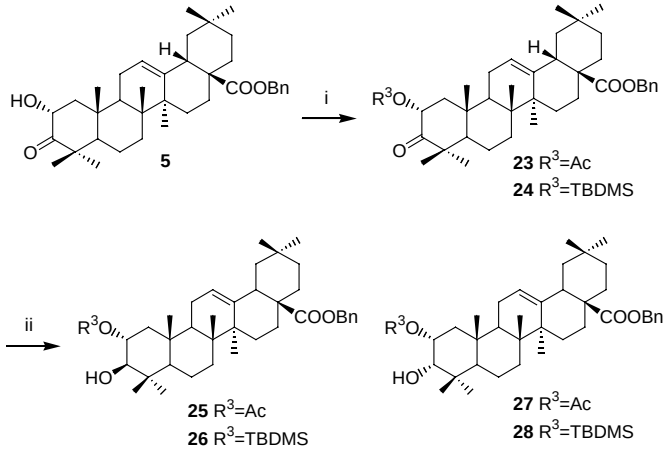
We examined how steric hindrance at C-2 affected stereoselectivity of the reduction of the C-3 carbony group by adding bulky substituents on 2α-hydroxyl group of **5**. In this regard, acetate **23** and silyl ether **24**



Scheme 1. Reagents and conditions: (i) K_2CO_3, BnCl, DMF, 50 °C; (ii) PCC, CH_2Cl_2; (iii) mCPBA, concd H_2SO_4 (cat), MeOH-CH_2Cl_2, 0 °C; (iv) $NaBH_4$, THF, 0 °C; (v) H_2, Pd/C, THF, rt; (vi) KOH, MeOH-DMF.



Scheme 3. Reagents and conditions: (i) $BrCH_2CH_2Br$ or $BrCH_2(CH_2)_2CH_2Br$, K_2CO_3, DMF, rt; (ii) for **16**: Et_3N, aqueous ethanol, reflux; for **17**–**22**: amine, K_2CO_3, DMF, rt.



Scheme 4. Reagents and conditions: (i) for **23**: Ac_2O, pyridine, rt; for **24**: TBDMSCl, imidazole, DMF, rt; (ii) $NaBH_4$, THF, 0 °C.

were prepared in high yields using the regular methods, respectively (Scheme 4). Our preliminary results showed that reduction of acetate **23** with $NaBH_4$ resulted in a similar stereoselectivity compared with reduction of **5**, furnishing 2α,3β-isomer **25** (75%) as the major product, together with 2α,3α-isomer **27** (14%) as a minor product. Even with the very bulky silyl ether **24**, the stereoselectivity of reduction was not significantly distinguished compared with that of reduction of **5**, and as a result, 2α,3β-isomer**26** (74%) was obtained as the major product, together with 2α,3α-isomer**28** (19%).

The synthesized maslinic acid derivatives were evaluated in the enzyme inhibition assay against rabbit muscle glycogen phosphorylase a which shared considerable sequence similarity with human liver GPa and was commercially available. As described previously,[14] 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 showed that most of the newly synthesized compounds exhibited inhibitory activity against rabbit muscle GPa with IC_{50} values in the range of 7–1707 μM (Table 1).

Our initial effort for lead optimization was focused on improving water solubility of the triterpenoids since it is well known that pentacyclic triterpenoids including **1** have very poor water solubility. Unfortunately, the incorporation of hydrophilic groups at C-28 side chain resulted in a significant decrease of potency (e.g., **16**, **17**, and **21**). SAR analysis for C-28 side chains indicated a strong preference for the hydrophobic groups (**12**, **14**, and **15**) over hydrophilic groups (**13**, **16**, and **20**). One hypothesis for this phenomenon is that the triterpenoids might bind to the inhibitor site (I-site) of GP, which is a hydrophobic binding pocket.[5] As an effort to identify the possible binding sites in GP for these triterpenoids, we performed molecular modeling studies, and the docking results showed a high probability of interaction between these triterpenoids and the inhibitor site of GP.[15] Furthermore, our previous studies showed that GP inhibition by **1** was synergistic with glucose,[10b] which was supportive of the above hypothesis since the I-site inhibitors were reported to be more potent in the presence of high glucose concentrations.[16] Certainly, in order to unanimously determine the GP-binding site for these triterpene inhibitors, an X-ray crystallographic study is needed.

Table 1. Rabbit muscle GPa inhibition assay results for maslinic acid derivatives

Compound	RMGPa IC_{50}[a] (μM)
1	28
5	29
6	nd[b]
7	66
8	144
9	30
10	393
11	66
12	19
13	1651
14	50
15	7
16	153
17	51
18	31
19	43
20	62
21	580
22	121
23	29
24	nd[b]
25	80
26	1707
27	63
28	580
Caffeine	114

[a] Values are means of three experiments.
[b] nd, not determined.

The effect of C-28 side-chain size could be clarified by a comparison of **14** and **15**. Compound **15** (IC_{50} = 7 μM) is much more potent than **14** (IC_{50} = 50 μM). The only structural difference between **14** and **15** is the length of carbon side chain linked to the C-28 carboxyl, indicating that the size of C-28 hydrophobic side chains affects the enzyme inhibitory activity.

Data analysis indicated no clear SAR for compounds based on structural modifications at C-2 and C-3 positions of **1**. In addition, no specific stereochemistry preferred by the enzyme could be inferred.

In summary, a series of maslinic acid derivatives have been synthesized and evaluated as novel GP inhibitors. Within this series of compounds, **15** was the most potent GPa inhibitor (IC_{50} = 7 μM). A clear preference for hydrophobic groups at C-28 is evident for enzyme inhibition, which raises a challenge for lead optimization based on **1** and related triterpenoids since good water solubility is usually preferred for drug design. A broader SAR study on triterpene class of GP inhibitors is ongoing in our laboratory and will be reported in due time.

Acknowledgments

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References and notes

1. Kurukulasuriya, R.; Link, J. T.; Madar, D. J.; Pei, Z.; Richards, S. J.; Rohde, J. J.; Souers, A. J.; Szczepankiewicz, B. G. *Curr. Med. Chem.* **2003**, *10*, 123.
2. (a) Oikonomakos, N. G. *Curr. Protein Pept. Sci.* **2002**, *3*, 561; (b) Somsák, L.; Nagy, V.; Hadady, Z.; Docsa, T.; Gergely, P. *Curr. Pharm. Des.* **2003**, *9*, 1177.
3. Tracey, W. R.; Treadway, J. L.; Magee, W. P.; Sutt, J. C.; McPherson, R. K.; Levy, C. B.; Wilder, D. E.; Yu, L. J.; Chen, Y.; Shanker, R. M.; Mutchler, A. K.; Smith, David M.; Flynn, A. H.; Knight, D. R. *Am. J. Physiol. Heart Circ. Physiol.* **2004**, *286*, H1177.
4. (a) Schnier, J. B.; Nishi, K.; Monks, A.; Gorin, F. A.; Bradbury, E. M. *Biochem. Biophys. Res. Commun.* **2003**, *309*, 126; (b) Geschwind, J.-F.; Georgiades, C. S.; Ko, Y. H.; Pedersen, P. L. *Expert Rev. Anticancer Ther.* **2004**, *3*, 449; (c) Laszlo, G. B.; Vay Liang, W. G.; Wai-Nang, P. L. *Pancreas* **2003**, *4*, 368.
5. (a) Lu, Z. J.; Bohn, J.; Bergeron, R.; Deng, Q. L.; Ellsworth, K. P.; Geissler, W. M.; Harris, G.; McCann, P. E.; Mckeever, B.; Myers, R. W.; Saperstein, R.; Willoughby, C. A.; Yao, J.; Chapman, K. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4125; (b) Wright, S. W.; Rath, V. L.; Genereux, P. E.; Hageman, D. L.; Levy, C. B.; McClure, L. D.; McCoid, S. C.; McPherson, R. K.; Schelhorn, T. M.; Wilder, D. E.; Zavadoski, W. J.; Gibbs, E. M.; Treadway, J. L. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 459; (c) Treadway, J. L.; Mendys, P.; Hoover, D. J. *Expert Opin. Invest. Drugs* **2001**, *10*, 439, and references therein; (d) Chrysina, E. D.; Kosmopolou, M. N.; Tiraidis, C.; Kardarakis, R.; Bischler, N.; Leonidas, D. D.; Hadady, Z.; Somsak, L.; Docsa, T.; Gergely, P.; Oikonomakos, N. G. *Protein Sci.* **2005**, *14*, 873.
6. (a) Taniguchi, S.; Imayoshi, Y.; Kobayashi, E.; Takamatsu, Y.; Ito, H.; Hatano, T.; Sakagami, H.; Tokuda, H.; Nishino, H.; Sugita, D.; Shimura, S.; Yoshida, T. *Phytochemistry* **2002**, *59*, 315; (b) Noriyasu, K.; Shinohara, G. Toshiyuki, I.U.S. Patent Application: US 2003153538, 2002.
7. Xu, H. X.; Zeng, F. Q.; Wan, M.; Sim, K. Y. *J. Nat. Prod.* **1996**, *59*, 643.
8. Montilla, M. P.; Agil, A.; Navarro, M. C.; Jimenez, M. I.; Garcia-Granados, A.; Parra, A.; Cabo, M. M. *Planta Med.* **2003**, *69*, 472.
9. Shinohara, H.; Shinohara, G.; Noriyasu, K. PCT Patent: WO 03011267, 2003.
10. (a) Sun, H. B; Wen, X. A.; Liu, J.; Zhang, L. Y.; Wang, S. Z.; Ni, P. Z. Chinese Patent Application: CN 200510038094.2, 2005; (b) Wen, X. A.; Sun, H. B.; Liu, J.; Wu, G. Z.; Zhang, L. Y.; Wu, X. M.; Ni, P. Z. *Bioorg. Chem. Med. Lett.* **2005**, *15*, 4944.
11. Takayama H.; Kitajima, M.; Ishizuka, T.; Seo S. U.S. Patent Application: US2005020681 A1, 2005.
12. (a) Dvoretzky, I.; Richtern, G. H. *J. Org. Chem.* **1950**, *15*, 1285; (b) Machin, P. J.; Hurst, D. N.; Bradshaw, R. M.; Blaber, L. C.; Burden, D. T.; Melaranget, R. A. *J. Med. Chem.* **1984**, *27*, 503.
13. Analytical data for 15: IR (KBr, cm^{-1}) 3572, 3427, 2947, 1726, 1464, 1383, 1258, 1159, 1051, 1036; ^1H NMR ($CDCl_3$, 300 MHz): δ 0.73, 0.83, 0.90, 0.93, 0.98, 1.03, 1.13 (each, 3H, s), 2.85 (1H, dd, *J* = 4.2, 13.7 Hz, H-18), 2.99 (1H, d, *J* = 9.5 Hz, H-3α), 3.43 (2H, t, *J* = 6.7 Hz, -CH_2Br), 3.70 (1H, m, H-2β), 4.05 (2H, t, *J* = 6.2 Hz, –COOCH_2–), 5.29 (1H, t, *J* = 3.5 Hz, H-12); EIMS: 629 $[M+Na]^+$. Analytical data for 20: IR (KBr, cm^{-1}) 3570, 3429, 2945, 1724, 1464, 1383, 1259, 1161, 1051; ^1H NMR ($CDCl_3$, 300 MHz): δ 0.74, 0.83, 0.90, 0.92, 0.98, 1.03, 1.13 (each, 3H, s), 1.04 (6H,

t, $J = 7.3$ Hz, $-N(CH_2CH_3)_2$), 2.48 (2H, t $J = 6.9$, $-CH_2N(CH_2CH_3)_2$), 2.55 (4H, q, $J = 7.1$ Hz, $-N(CH_2CH_3)_2$), 2.89 (1H, dd, $J = 4.0$, 14.2 Hz, H-18), 2.98 (1H, d, $J = 9.4$ Hz, H-3α), 3.69 (1H, m, H-2β), 4.02 (2H, m, $-COOCH_2-$), 5.28 (1H, br s, $J = 3.5$ Hz, H-12); EIMS: 600 $[M+H]^+$.

14. Engers, H. D.; Shechosky, S.; Madsen, N. B. *Can. J. Biochem.* **1970**, *48*, 746.
15. Manuscript in preparation.
16. (a) Kasvinsky, P. J.; Shechosky, S.; Fletterick, R. J. *J. Biol. Chem.* **1978**, *253*, 9102; (b) Ercan-Fang, N.; Nuttall, F. Q. *J. Pharmcol. Exp. Ther.* **1997**, *280*, 1312.