SYNTHETIC STUDIES OF MANGOSTIN DERIVATIVES WITH AN INHIBITORY ACTIVITY ON PDGF-INDUCED HUMAN AORTIC SMOOTH CELLS PROLIFERATION

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Abstract — The mangostin derivatives 3—10, were synthesized by halogenation, electrochemical oxidation, and mCPBA oxidation of α- and γ-mangostins (1, 2). Among them, the hydroxyl 9 and the benzopyran 10 derivatives produced by mCPBA, showed remarkable antiproliferative activities against human aortic smooth muscle cells (HASMC) induced by platelet-derived growth factor (PDGF).

INTRODUCTION

Natural products possessing the xanthone-skeleton are found in a wide range of plants, and exhibit a variety of biological activities. We have performed synthetic and biological studies of α-mangostin (1), a representative naturally occurring xanthone. Although xanthones are generally obtained in large quantities, there have been no previous reports of chemical modification studies with the aim of production of new bioactive substance, with the exception of our previous report. Given such chemical transformation, it is expected that to be possible to construct of novel bioactive substances starting from naturally abundant sources. Indeed, we proposed such a possibility by acquiring mangostin congeners with remarkable inhibitory activity against acidic sphingomyelinase.

This paper is dedicated to Professor Emeritus Keiichiro Fukumoto in celebration of his 75th birthday.
As part of our ongoing synthetic project mentioned above, we have carried out chemical modification of α- and γ-mangostins (1, 2) by halogenation, phenolic oxidation, and mCPBA oxidation to examine the antiproliferative activity against HASMC, which is accumulated in the endothelium with foam cells derived from LDL cholesterol comprising arteriosclerotic plaques. The grown and hypertrophic plaque will reduce and block blood flow leading to atherosclerosis, which will cause brain infarction and cardiac infarction. HASMC growth is promoted by growth factors, such as PDGF. Accordingly, suppression of HASMC-growth caused by PDGF may be useful to prevent atherosclerosis. Here, we report chemical modification of the mangostin derivatives from 1 and 2 and their antiproliferative activity against HASMC.

RESULTS AND DISCUSSION

Synthesis of halogenated mangostin derivatives

In addition to a scaffold leading to diverse functionalities, we are interested in the biological effects of halogens. Halogenated mangostin derivatives were synthesized as shown in Scheme 1. Treatment of 1 with NBS or NCS effected selective halogenation at the C-4 position to give 3a and 3b, respectively, in moderate yields, along with the O-methylated derivatives 4a and 4b prepared from 3 by the standard procedure.

![Scheme 1](image)

**Scheme 1.** Reagents and conditions: (a) NBS or NCS, THF, 3a; 51%, 3b; 46% (b) MeI, K₂CO₃, DMF, 4a; 67%, 4b; 77%

Phenolic oxidation of γ-mangostin

As part of our synthetic investigation employing electroorganic chemistry toward biologically active
substances, 2 was oxidized to understand the chemical features of the phenol functionalities (Scheme 2). Direct anodic oxidation [constant current electrolysis (C.C.E.); glassy carbon beaker as an anode; platinum wire as a cathode; LiClO₄ as a supporting salt] of 2 in MeOH gave the corresponding methoxydienone 5 in 51% yield, which on reaction with MeI-K₂CO₃ gave 6. Direct anodic oxidation in an EtOH solution yielded no reaction due to preferential oxidation of the solvent. The unsuccessful anodic oxidation prompted us to examine the electrochemically generated hypervalent iodo benzene species, which is produced from PhI under the anodic oxidation conditions using CF₃CH₂OH as a solvent. Although the generated oxidant was used without isolation, the active species may be bis(2,2,2-trifluoroethoxy)phenyliodine(III). Reaction of 2 with the oxidant in the presence of EtOH or nPrOH effected nucleophilic attack of the solvent to produce the expected dienones 7a and 7b.

![Scheme 2](image)

**Scheme 2. Reagents and conditions:** (a) C.C.E. (0.3 mA/cm²), 2 F/mol, MeOH, 51%. (b) MeI, K₂CO₃, DMF, 66%. (c) Oxidized iodobenzene, EtOH or nPrOH, 7a; 36%, 7b; 41%.

**Oxidation of the mangostin derivative 8**

Among the reaction conditions examined for structural conversion of the stable xanthone skeleton, we observed that mCPBA oxidation of 8 produced by two-step procedure from a mixture of mangostins with mCPBA, gave 9 and 10, along with a complex mixture (Scheme 3). While 9 underwent hydroxylation at the C-4 position similar to halogenation, the right aromatic ring of 8 was opened oxidatively to give the benzopyran 10, through a plausible mechanism depicted in Scheme 3.

**Biological Activity**

The inhibitory activities of natural 1 and 2, along with 3—7, 9, and 10 against PDGF-induced HASMC proliferation were examined by the [³H] thymidine incorporation procedure. In contrast to 3—7, which showed weak to moderate activities, compounds 9 and 10 produced by mCPBA-mediated oxidation exhibited remarkable inhibitory activities at a concentration of 1 μM (Figure 2(a)). Their
concentration-dependent inhibitory effects were confirmed at 0.1, 1, and 10 μM, and 9 with the most effective activity showed an IC₅₀ 0.1 μM (Figure 2 (b)). Unfortunately, the reason why the hydroxyl function of 9 contributed higher inhibitory activity than the Br-function of 3a remains unclear.

Scheme 3. Reagents and conditions: (a) MeI, K₂CO₃, DMF, 40 °C. (b) H₂, Pd-C, MeOH, 98%. (c) mCPBA, CH₂Cl₂, 9; 12%, 10; 19%.

Figure 2. Inhibitory activity on PDGF-stimulated HASMC proliferation
CONCLUSIONS
Chemical modification of natural 1 and 2 was carried out to provide halogenated, oxidized compounds 3—10. Compounds 9 and 10 exhibited potent inhibitory activities on PDGF-mediated HASMC proliferation. Further investigations to determine the structure-activity relationship of the inhibitory activities of the xanthone derivatives against HASMC are currently in progress in our laboratory.

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REFERENCES (AND NOTES)
6. A preliminary work was reported: see, Y. Nishihama, Y. Amano, T. Ogamino, and S. Nishiyama, Electrochem., 2006, 74, 609.
7. Structures of 5 and 10 were determinate by single X-ray crystallographic analysis. The detailed results will be reported elsewhere, owing to their inadequate R factors.
9. The yields were not optimized.
10. $[^3]$H-Thymidine Incorporation Assay: Cell proliferation was determined by $[^3]$H-thymidine incorporation. HASMC were incubated for 20 hours with or without PDGF-BB (20 ng/mL) and indicated concentrations of mangostins and its derivatives and then pulse-labeled with 1 μCi/mL of $[^3]$H-thymidine for 4 hours. Cells were harvested using a Universal Harvester (Perkin Elmer), and then transferred to a GF/C filter (Perkin Elmer). The filter was dried and counted in scintillation fluid using a Microplate Scintillation and Luminescence Counter-Topcount NXT (Perkin Elmer).
11. Compounds details: compound 3a; IR (film) 3486, 2919 and 1602 cm$^{-1}$; δH (ACETN), 1.63 (6H, s),
1.77 (3H, s), 1.82 (3H, s), 3.40 (2H, d, $J = 7.2$ Hz), 4.14 (2H, d, $J = 6.8$ Hz), 5.22 (1H, t, $J = 7.2$ Hz), 5.28 (1H, t, $J = 6.8$ Hz), 6.89 (1H, s), 13.95 (1H, s); $\delta_C$ (ACETN), 17.9, 18.3, 22.7, 25.8, 26.0, 26.3, 86.7, 101.1, 104.5, 111.5, 111.6, 122.7, 124.1, 129.0, 131.3, 131.9, 141.9, 151.5, 152.4, 153.1, 158.4, 160.4, 182.7  

3b; $\delta_H$ (CDCl$_3$), 1.67 (3H, s), 1.69 (3H, s), 1.80 (3H, s), 1.81 (3H, s), 3.42 (2H, d, $J = 7.3$ Hz), 3.80 (3H, s), 4.06 (2H, d, $J = 6.8$ Hz), 5.22 (1H, t, $J = 5.9$ Hz), 5.25 (1H, t, $J = 7.3$ Hz), 6.34 (1H, br), 6.38 (1H, br), 6.93 (1H, s), 13.60 (1H, s); $\delta_H$ (CDCl$_3$), 1.68 (3H, s), 1.69 (3H, s), 1.81 (3H, s), 1.85 (3H, s), 3.44 (2H, d, $J = 7.3$ Hz), 3.81 (3H, s), 3.92 (3H, s), 4.11 (2H, d, $J = 6.8$ Hz), 5.21 (1H, t, $J = 6.8$ Hz), 5.24 (1H, t, $J = 7.3$ Hz), 6.89 (1H, s), 13.68 (1H, s); $\delta_C$ (ACETN), 17.9, 18.0, 21.6, 25.8, 25.9, 38.5, 53.8, 83.0, 93.8, 104.6, 108.9, 110.6, 113.2, 114.3, 120.8, 136.1, 137.7, 151.8, 154.8, 159.5, 160.0, 161.3, 178.4, 199.1; 6; $\delta_H$ (CDCl$_3$), 1.43 (3H, s), 1.52 (3H, s), 1.66 (3H, s), 1.77 (3H, s), 2.84 (1H, dd, $J = 8.6, 13.2$ Hz), 3.10 (3H, s), 3.25 (1H, dd, $J = 7.3, 13.2$ Hz), 3.34 (2H, d, $J = 6.8$ Hz), 3.85 (3H, s), 3.89 (3H, s), 4.77 (1H, t, $J = 8.0$ Hz), 5.20 (1H, t, $J = 6.8$ Hz), 6.17 (1H, s), 6.37 (1H, s), 13.05 (1H, s); 7a; $\delta_H$ (CDCl$_3$), 1.11 (3H, t, $J = 6.9$ Hz), 1.37 (3H, s), 1.47 (3H, s), 1.70 (3H, s), 1.77 (3H, s), 2.80 (1H, dd, $J = 7.9$ Hz, 13.5 Hz), 3.04-3.10 (1H, m), 3.20-3.34 (2H, m), 3.38 (2H, d, $J = 6.6$ Hz), 4.65 (1H, t, $J = 7.9$ Hz), 5.21 (1H, t, $J = 6.9$ Hz), 6.34 (1H, br), 6.37 (1H, s), 13.38 (1H, s); $\delta_C$ (CDCl$_3$), 15.7, 18.0, 21.6, 25.8, 25.9, 38.8, 61.9, 82.2, 90.1, 93.8, 108.7, 110.5, 113.7, 114.4, 121.0, 135.8, 137.4, 152.2, 154.7, 159.6, 159.9, 161.2, 171.5, 178.3, 199.7; 7b; $\delta_H$ (CDCl$_3$), 0.34 (3H, t, $J = 6.8$ Hz), 1.45 (3H, s), 1.54 (2H, m), 1.58 (2H, m), 1.77 (3H, s), 1.85 (3H, s), 2.88 (1H, dd, $J = 7.3$ Hz, 12.7 Hz), 2.96 (1H, m), 3.22 (1H, m), 3.44 (1H, dd, $J = 7.3$ Hz, 12.7 Hz), 3.46 (2H, d, $J = 6.3$ Hz), 4.71 (1H, t, $J = 7.3$ Hz), 5.28 (1H, t, $J = 6.3$ Hz), 6.42 (1H, s), 6.47 (1H, s), 6.50 (1H, br), 6.88 (1H, br), 13.45 (1H, s); $\delta_C$ (CDCl$_3$), 10.5, 18.0, 21.6, 23.2, 25.8, 25.9, 38.6, 68.2, 82.0, 93.8, 104.7, 108.8, 110.5, 114.0, 114.4, 121.7, 135.9, 137.5, 151.7, 154.8, 159.6, 159.6, 161.2, 178.4, 199.5; 8; IR (film) 2954, 1650 and 1614 cm$^{-1}$; $\delta_H$ (CDCl$_3$), 0.92 (6H, d, $J = 6.8$ Hz), 0.98 (6H, d, $J = 6.8$ Hz), 1.35 (2H, m), 1.46 (2H, m), 1.59 (1H, m), 1.76 (1H, m), 2.62 (2H, t, $J = 8.0$ Hz), 3.35 (2H, t, $J = 7.8$ Hz), 3.78 (3H, s), 3.84 (3H, s), 3.85 (3H, s), 3.89 (3H, s), 6.49 (1H, s), 6.63 (1H, s); $\delta_C$ (CDCl$_3$), 21.1, 22.5, 22.6, 24.8, 28.4, 28.7, 39.1, 40.4, 55.7, 55.8, 61.0, 61.9, 93.9, 97.4, 110.6, 114.6, 121.7, 138.1, 143.5, 154.1, 156.1, 156.6, 158.3, 162.0, 175.9; HRMS (EI) calcd for C$_{27}$H$_{36}$O$_6$ (M$^+$) 456.2512, found: $m/z$ 456.2512; 9; mp 156-157°C (hexane-EtOAc); IR (disk):
3311, 2954, 1592 and 1454 cm\(^{-1}\); \(\delta_H\) (CDCl\(_3\)), 0.94 (6H, d, \(J = 6.4\) Hz), 0.96 (6H, d, \(J = 6.4\) Hz), 1.43 (4H, m), 1.62 (2H, m), 1.75 (1H, m), 2.63 (2H, t, \(J = 8.0\) Hz), 3.32 (2H, t, \(J = 8.0\) Hz), 3.79 (3H, s), 3.82 (3H, s), 3.88 (3H, s), 3.95 (3H, s), 5.65 (1H, br), 6.73 (1H, s); \(\delta_C\) (CDCl\(_3\)), 22.0, 22.5, 22.6, 25.0, 28.6, 28.8, 39.9, 40.4, 55.9, 61.1, 61.2, 62.2, 97.5, 113.3, 114.4, 125.9, 132.9, 139.4, 143.3, 143.8, 148.9, 150.6, 153.9, 157.0, 176.2; HRMS; \(m/z\) 472.2445 (calcd for C\(_{27}H_{36}O_7\) M\(^+\), 472.2461); 10; IR (film) 2954, 1776 and 1635 cm\(^{-1}\); \(\delta_H\) (CDCl\(_3\)), 0.88 (6H, d, \(J = 6.4\) Hz), 0.93 (6H, d, \(J = 6.4\) Hz), 1.37 (2H, m), 1.66 (4H, m), 2.73 (2H, t, \(J = 7.3\) Hz), 3.27 (2H, t, \(J = 7.8\) Hz), 3.77 (3H, s), 3.80 (3H, s), 3.88 (3H, s), 3.91 (3H, s), 5.57 (1H, s), 6.47 (1H, s); \(\delta_C\) (CDCl\(_3\)), 22.4, 22.6, 24.7, 27.6, 28.6, 32.0, 55.8, 55.9, 56.6, 61.1, 89.1, 97.5, 115.4, 132.4, 139.0, 144.6, 151.1, 152.9, 154.0, 156.9, 161.6, 171.3, 199.4.