Pergamon

Bioorganic & Medicinal Chemistry 10 (2002) 1189–1196

BIOORGANIC & MEDICINAL CHEMISTRY

Syntheses of Ferulic Acid Derivatives and Their Suppressive Effects on Cyclooxygenase-2 Promoter Activity

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Received 31 August 2001; accepted 28 October 2001

Abstract—Novel ferulic acid derivatives in which feruloyl groups were attached to the hydroxyl groups of *myo*-inositol 1,3,5-orthoformate derivatives were synthesized. These feruloyl-*myo*-inositols suppressed the cyclooxygenase-2 (COX-2) promoter activity in a concentration-dependent manner. Among the examined compounds, compound **9** showed the highest activity. A treatment with 100 μM of compound **9** for 24 h resulted in a 50% decrease of COX-2 promoter activity without marked cytotoxicity. Both the molecular structure in which two ferulic acid moieties are facing each other and the molecular hydrophobicity may be essential for the suppression of COX-2 promoter activity.

Introduction

Cyclooxygenase (COX) is one of the key enzymes in the biosynthesis of proinflammatory prostaglandins. Two isoforms of COX are known.[1] Cyclooxygenase-1 (COX-1) is constitutively expressed to maintain physiological functions, while cyclooxygenase-2 (COX-2) is an inducible enzyme that is upregulated during inflammation and colorectal tumor formation.[2–9] Recent studies have suggested that the overexpression of COX-2 and the resultant over-production of prostaglandins might be involved in the development of colon cancer.[10] Therefore, selective inhibitors of COX-2 are able to become candidates of chemopreventive agents against the development of cancer in the colon and other organs.

For selective inhibitors of COX-2, there are many reports.[11] Although the suppression of COX-2 expression at the gene level has recently attracted much attention, the suppressive agents for COX-2 promoter activity have been scarcely reported, except polyphenolic compounds such as genistein, quercetin and (−)-epigallocatechin gallate.[12,13] However, it is difficult for these natural cancer chemopreventive agents to be put to practical use, because the amounts of these compounds are very small in plants. Therefore, the synthetic compounds are required for the practical use as cancer chemopreventive agents.[14,15]

Recently, we have developed an efficient and practical method for the mass production of ferulic acid (**1a**) from the oily component of rice bran.[16] Since then, compound **1a** and related compounds have attracted considerable attention in the field of chemopreventive study. For example, the ferulic acid derivative, ethyl 3-(4′-geranyloxy-3′-methoxyphenyl)-2-propenoate (EGMP, **1b**),[16b] in which geranyl group is attached to the phenolic hydroxyl group of ethyl ferulate, shows a suppressive effect on the formation of a colonic tumor marker in rats.[17,18] In addition, it is shown that EGMP attenuates the expression of the COX-2 protein.[19] Then, the design and synthesis of novel ferulic acid derivatives having the suppressive activity of COX-2 gene expression are required.

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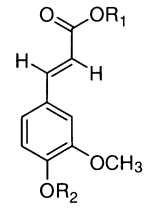
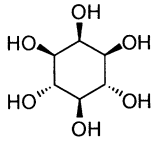
0968-0896/02/$ - see front matter
PII: S0968-0896(01)00386-8



R1 R2
1a : H H
1b : ethyl geranyl
1c : ethyl H



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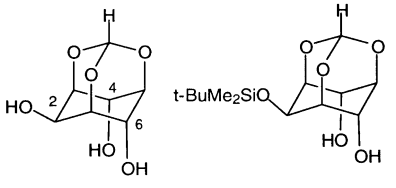
Among these approaches, we have much attention the compounds in which compound **1a** combines with *myo*-inositol (**2**). *myo*-Inositol is also obtained from rice bran, and it plays an important role in biological systems. D-*myo*-Inositol-1,4,5-trisphosphate is found to act as an intracellular second messenger for calcium mobilization.[20,21] *myo*-Inositol hexaphosphate (IP_6) is shown to have an anticancer action in a variety of experimental tumor models.[22] Ferulic acid occurs usually as various ester or amide derivatives in natural products,[23] but the ester compounds consisting of ferulic acid and *myo*-inositol have not yet been discovered in nature.

In a previous paper, we reported the synthesis of seven ester compounds consisting of ferulic acid and *myo*-inositol and their inhibitory effect on 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced superoxide (O_2^-) generation by the use of differentiated HL-60 cells.[24] In this study, we found that only 3,4,5,6-tetra-*O*-acetyl-1,2-di-*O*-[3-(4′-acetoxy-3′-methoxyphenyl)-2-propenoyl]-*myo*-inositol showed a distinct inhibitory activity. This result suggests that the architecture having two feruloyl groups is essential for the inhibitory activity. In addition, it is shown that the hydrophobicity of the feruloyl-*myo*-inositol derivative also plays a crucial role on suppression of O_2^- generation.



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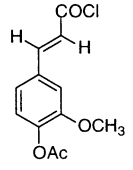

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On the basis of these backgrounds, we newly designed compounds in which two ferulic acid moieties were facing each other by the use of a *myo*-inositol 1,3,5-orthoformate skeleton. In the present study, we report the preparation of these novel feruloyl-*myo*-inositol derivatives and their suppressive ability of COX-2 promoter activity through the β-galactosidase reporter gene assay system in human colon cancer DLD-1 cells.[13,25]

Results and Discussion

Chemistry

myo-Inositol 1,3,5-orthoformate (**3**) is synthesized by one step from *myo*-inositol.[26] 2-*O*-*tert*-Butyldimethylsilyl-*myo*-inositol 1,3,5-orthoformate (**4**) is prepared from compound **3** by using a common method.[27] Ferulic acid (**1a**) was converted into 3-(4′-acetoxy-3′-methoxyphenyl)-2-propenoyl chloride (**5**) via two steps.[28]

The synthesis of ferulic acid derivatives, in which feruloyl groups are attached to the hydroxyl groups of *myo*-inositol 1,3,5-orthoformate derivatives, is summarized in Scheme 1.

The reaction of **3** with **5** was carried out in the presence of a catalytic amount of 4-(dimethylamino)pyridine (DMAP) in anhydrous pyridine at 90 °C to give compound **6** in 80% yield. The deacetylation of **6** with $NH_2NH_2 \cdot H_2O$ in $CHCl_3$–MeOH (7:3) at room temperature gave compound **7** in 97% yield. The reaction of **4** with **5** was carried out in anhydrous pyridine at room temperature in the presence of DMAP. When the substrate molar ratio of **5** to **4** was 3.0, the reaction gave compound **8** in 88% yield. When the molar ratio of **5** to **4** was 1.5, the reaction afforded the product **11** in 30% yield. Compounds **8** and **11** were deacetylated by the use of hydrazine to produce compounds **9** and **12** in 93 and 88% yield, respectively. Compound **9** was treated with n-$Bu_4NF \cdot 3H_2O$ in THF to afford compound **10** in 98% yield.

Compound **8** was recrystallized from acetone–*n*-hexane to give prism crystals. We confirmed the molecular structure of **8**, in which two ferulic acid moieties were facing each other, by a single crystal X-ray analysis (Fig. 1). The molecular structures of other compounds were established by NMR,

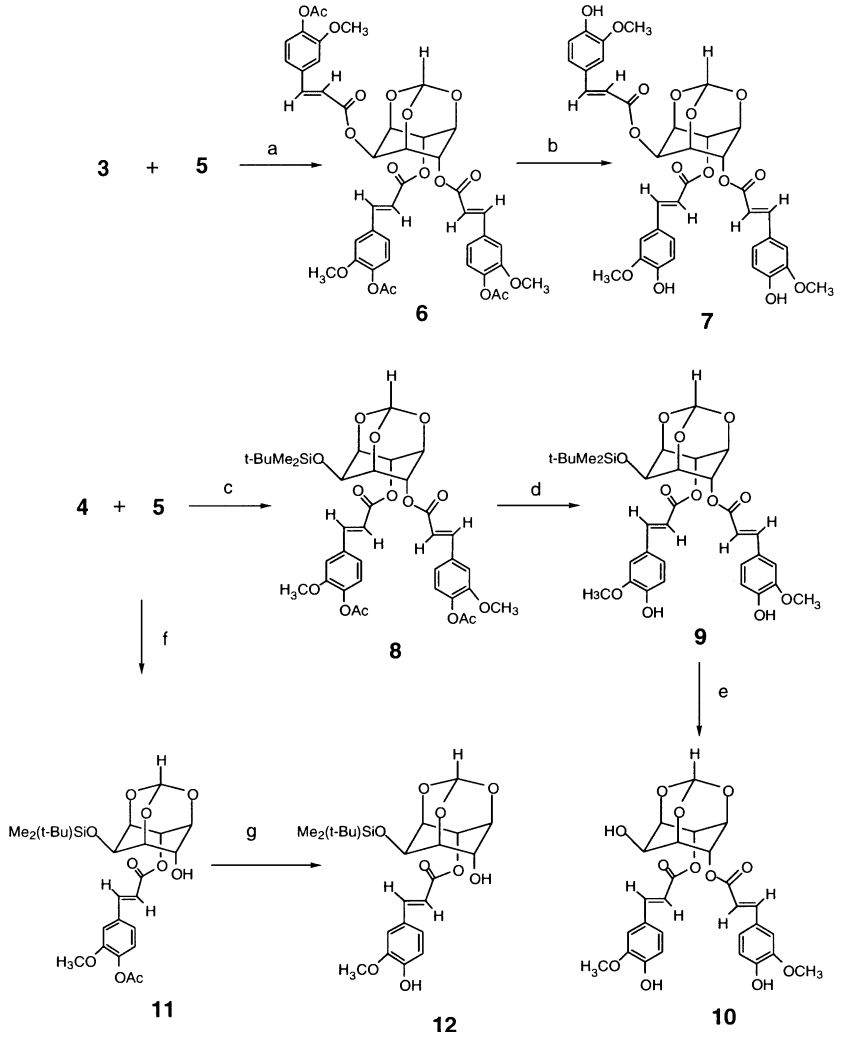
For comparison, we also prepared feruloyl-*myo*-inositol compounds in which the orthoformate group was removed from compounds **7** and **9** (Scheme 2).

Compounds **7** and **9** were treated with 80% aqueous trifluoroacetic acid at room temperature to produce 2,4,6-tris-*O*-[3-(4′-hydroxy-3′-methoxyphenyl)-2-propenoyl]-*myo*-inositol (**13**) and 4,6-di-*O*-[3-(4′-hydroxy-3′-methoxyphenyl)-2-propenoyl]-*myo*-inositol (**14**) in 43 and 50% yield, respectively.

Biological Evaluation

We examined the COX-2 promoter activity of novel feruloyl-*myo*-inositol derivatives, **6**, **7**, **8**, **9**, **10**, **12**, **13** and **14** using the β-galactosidase reporter gene assay system in human colon cancer DLD-1 cells. For comparison, we also examined the COX-2 promoter activity of compounds **1a**, **1b**, **4** and ethyl ferulate (**1c**).[16]

As shown in Figure 2, it was found that the feruloyl-*myo*-inositol orthoformate derivatives suppressed COX-2 promoter activity.



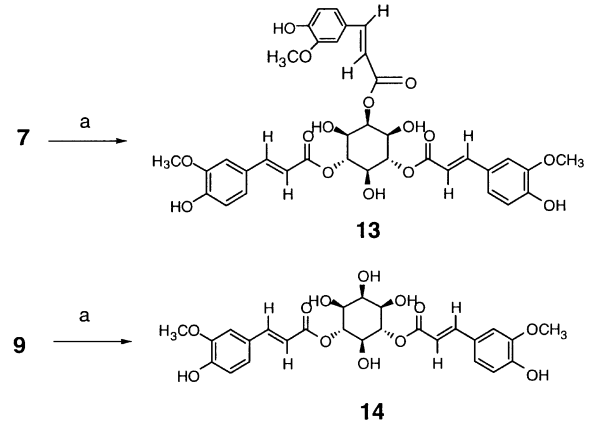
Scheme 1. (a) Py, DMAP, 90 °C, 67%; (b) $NH_2NH_2 \cdot H_2O$, 7:3 $CHCl_3$–MeOH, rt, 100%; (c) Py, DMAP, rt, 88%; (d) $NH_2NH_2 \cdot H_2O$, 7:3 $CHCl_3$–MeOH, rt, 93%; (e) n-$Bu_4NF \cdot 3H_2O$, THF, 98%; (f) Py, DMAP, rt, 30%; (g) $NH_2NH_2 \cdot H_2O$, 3:2 $CHCl_3$–MeOH, rt, 88%.

Among the examined compounds, compound **9** showed the highest activity. As shown in Figure 3(B), compound **9** suppressed COX-2 promoter activity in a concentration-dependent manner. A treatment with 100 μM of compound **9** for 24 h resulted in a 50% decrease of COX-2 promoter activity without marked cytotoxicity.[29]

In addition, it became clear that compound **9** had a suppressive ability of the COX-2 promoter activity comparable to that of the synthetic cancer chemopreventive agents **1b** (Fig. 2). On the other hand, it is known that the natural polyphenolic compounds such as quercetin, genistein and resveratol suppress the COX-2 promoter activity in a similar assay system.[13] Treatments with 20 μM quercetin, 40 μM genistein and 100 μM resveratol for 48 h resulted in 42, 58 and 66% decrease in the COX-2 promoter activity, respectively.[13]

Therefore, these results show that the COX-2 promoter activity of compound **9** is comparable to that of these natural polyphenolic compounds.

In contrast, compounds **1a**, **1c** and **4** showed no activity, and both *myo*-inositol ferulate derivatives **13** and **14** exhibited a low activity (Fig. 2). Therefore, it is important that the molecular structure consisting of ferulic acid moiety and *myo*-inositol 1,3,5-orthoformate moiety is necessary for the suppression of COX-2 promoter activity. Moreover, the COX-2 promoter activity of compounds **7** and **9** was higher than that of compound **12**. Hence, the molecular structure in which two ferulic acid moieties are facing each other is also important for the suppressive activity.



Scheme 2. (a) 80% CF_3COOH aq, rt.

In addition, compound **8**, in which the phenolic hydroxyl groups of ferulic acid moiety in compound **9** were acetylated, showed lower activity than compound **9**. Similarly, compound **7** showed higher activity in comparison with compound **6**. These results suggest that the phenolic hydroxyl group plays an important role on the suppression of COX-2 promoter activity.

Murakami et al. reported that the hydrophobicity of alkyl ferulates plays a crucial role on suppression of O_2^- generation; the relationship between the hydrophobicity of alkyl ferulates and the suppression potency was explained by their cellular incorporating rate from a cellular uptake experiment.[19] Moreover, it was shown that the hydrophobicity of feruloyl-*myo*-inositol derivative also plays a crucial role on suppression of O_2^- generation.[24]

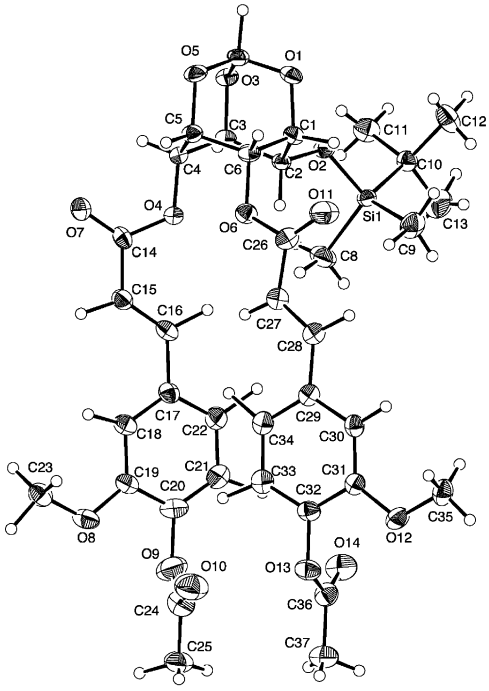


Figure 1. ORTEP drawing of **8**.

In the present study, it was found that compound **10** showed lower activity than that of compound **9**. The structural difference between **9** and **10** is whether the *tert*-butyldimethylsilyl group exists in the compound or not. Their contrasting activity difference may be attributable to their molecular hydrophorbicity. Hence, we also regard that the cellular incorporating is one of the important events on the suppression of COX-2 promoter activity.

Conclusion

We have newly designed compounds in which two ferulic acid moieties are facing each other by the use of a *myo*-inositol 1,3,5-orthoformate skeleton. Among these compounds, it was found that compound **9** showed a distinct suppressive effect on the COX-2 promoter activity comparable to **1b** and natural polyphenols.

The recent progress of molecular biology makes it possible to diagnose the susceptibility of cancer. Therefore, the cancer chemopreventive study and cancer chemopreventive agents will be increasingly attracted much attention.

A lot of natural products are reported as cancer chemopreventive agents, however, it is difficult for these cancer chemopreventive agents to be used practically,

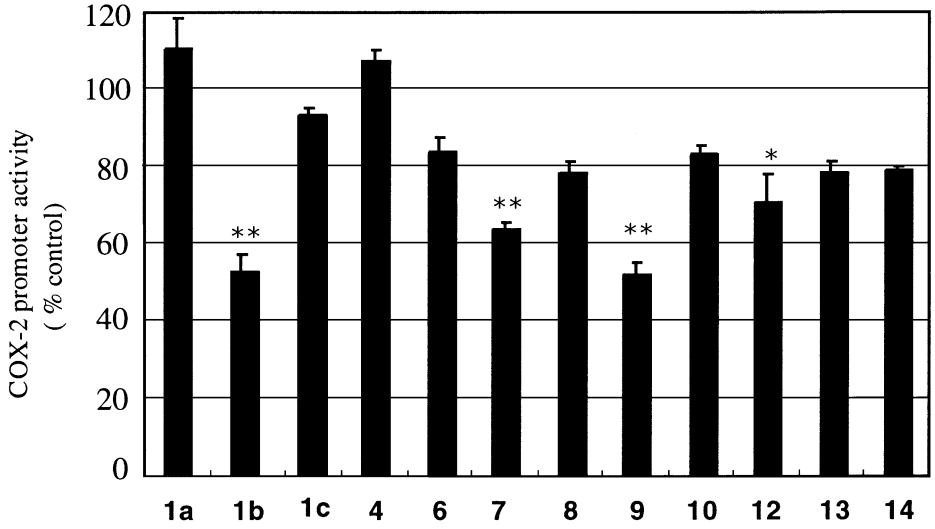


Figure 2. The effects of feruloyl-*myo*-inositol orthoformate derivatives on COX-2 promoter activity in DLD-1 cells. DLD-1/COX-2-B2-βGal-BSD cells were cultured in medium containing 5% FBS. The cells were precultured for 24 h and plied to the culture with test compounds **1a**, **1b**, **1c, 4, 6**, **7**, **8**, **9**, **10**, **12**, **13** and **14** for 24 h at 100 μM. The β-galactosidase activity in untreated negative control DLD-1/B2-βGal-BSD cells was set at 0, and that in DLD-1/COX-2-B2-βGal-BSD cells was set at 100%. β-Galactosidase reporter gene activity was normalized for viable cell numbers assessed by MTT assay. Data are shown as means ± SD values from three independent experiments. * $p < 0.01$; ** $p < 0.001$ versus control in Student's *t*-test.

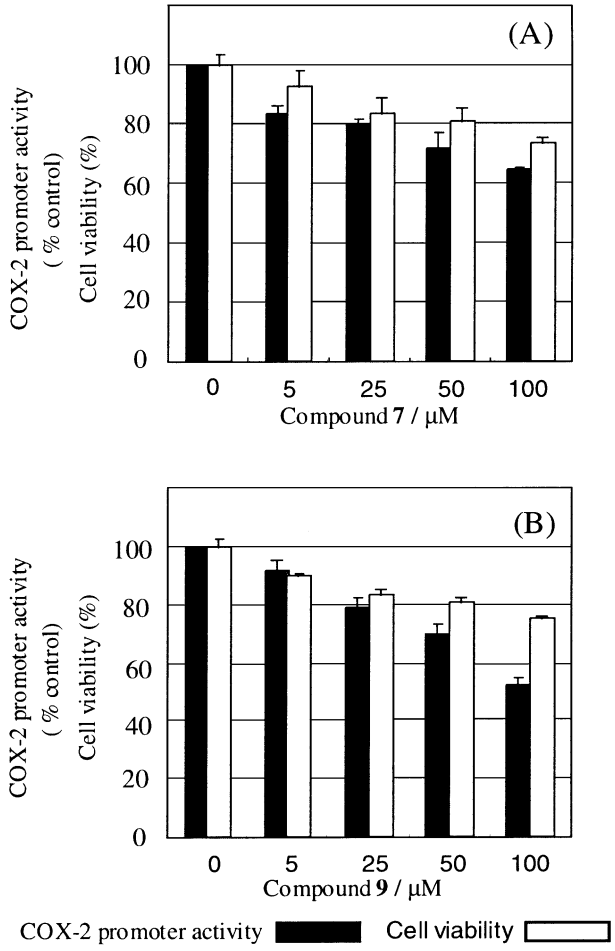


Figure 3. Suppression of COX-2 promoter activity in DLD-1 cells: (A) compound 7; (B) compound **9**.

because the amounts of these agents are very small in plants. Therefore, the synthetic compounds are in great demand for cancer chemopreventive agents. From that point of view, the synthetic feruloyl-*myo*-inositol 1,3,5-orthoformate derivatives are fascinating compounds as a kind of cancer chemopreventive agent. We believe that the present results will provide useful informations for the cancer chemopreventive study and the development of cancer chemopreventive agents.

Experimental

Chemistry

The ^{1}H NMR (400 MHz) spectra were recorded on a Varian unity-plus 400 spectrometer using tetramethylsilane as internal standard. Chemical shifts are reported in δ (ppm) and coupling constants (*J*) are given in Hertz. FT-IR spectra were recorded on a Shimadzu FT-IR8200D instrument using a diffuse reflectance cell. Melting points were measured in capillary tubes with a Yanagimoto micro melting point apparatus, and are uncorrected. Elemental analyses were performed on a Perkin–Elmer 2400II. Compound purity was checked by TLC on Silica Gel 60 F254 (E. Merck) with detection by charring with phosphomolybdic acid (10% in EtOH solution). Column chromatography was performed on Silica Gel, Wakogel C-200 (Wako Pure Chemical Industry). Ferulic acid and *myo*-inositol were provided by Tsuno Food Industrial Co., Ltd. Ferulic acid was recrystallized from ethanol. *myo*-Inositol was dried under reduced pressure in a drying oven. *myo*-Inositol 1,3,5-orthoformate (**3**), 2-*O*-*tert*-butyldimethylsilyl-*myo*-inositol 1,3,5-orthoformate (**4**) and 3-(4′-acetoxy-3′-methoxyphenyl)-2-propenoyl chloride (**5**) were prepared according to the literatures.[26–28] Other chemicals were commercial products and used without further purification. Solvents were reagent grade and in most cases dried prior to use.

2,4,6-Tris-*O*-[3-(4′-acetoxy-3′-methoxyphenyl)-2-propenoyl]-*myo*-inositol 1,3,5-orthoformate (6). The acid chloride **5** (7.64 g, 30.0 mmol) was added to a solution of **3** (0.95 g, 5.0 mmol) and catalytic amount of DMAP in anhydrous pyridine (50 mL). The mixture was stirred for 3 h at 90 °C and the reaction was quenched by adding water. The solvent was evaporated under reduced pressure and the residue was dissolved in 80 mL CH_2Cl_2. The organic layer was washed successively with saturated aqueous $NaHCO_3$, and brine. After the organic layer was dried over Na_2SO_4, the solvent was removed under reduced pressure. The residue was chromatographed (silica gel, *n*-hexane/ethyl acetate, 1:1) to give **6** (2.50 g, 80%) as a white solid. Mp 175–177 °C; IR (KBr) ν 3070, 3010, 2939, 2841, 1766, 1720, 1636, 1601, 1508, 1419, 1369, 1259, 1155, 1003, 949, 903, 833 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.77 (d, 1H, *J* = 16.0 Hz, CH=), 7.67 (d, 2H, *J* = 16.0 Hz, CH=), 6.87–7.27 (m, 9H, aromatic), 6.58 (d, 1H, *J* = 16.0 Hz, CH=), 6.33 (d, 2H, *J* = 16.0 Hz, CH=), 5.71 (d, 1H, *J* = 1.2 Hz, CH), 5.68 (t, 2H, *J* = 4.0 Hz, H-4 and -6), 5.54 (m, 1H, H-2), 4.89 (m, 1H, H-5), 4.56 (m, 2H, H-1 and -3), 3.88 (s, 3H, OCH_3), 3.71 (s, 6H, OCH_3), 2.33 (s, 3H, acetyl), 2.32 (s, 6H, acetyl) ppm. Anal. calcd for $C_{43}H_{40}O_{18}$: C, 61.14; H, 4.77; found: C, 61.03; H, 4.73.

2,4,6-Tris-*O*-[3-(4′-hydroxy-3′-methoxyphenyl)-2-propenoyl] - *myo* - inositol 1,3,5 - orthoformate (7). The compound **6** (0.78 g, 0.92 mmol) was dissolved in a mixture of $CHCl_3$ (23 mL) and MeOH (10 mL) and treated with $NH_2NH_2{\cdot}H_2O$ (144 μL, 2.8 mmol). After being stirred at room temperature for 3 h, the reaction mixture was diluted with $CHCl_3$ and successively washed with saturated aqueous $KHSO_4$, saturated aqueous $NaHCO_3$, and brine. The organic layer was dried over anhydrous Na_2SO_4. The solvent was evaporated and the residue was recrystallized from EtOAc/*n*-hexane to provide **7** as colorless crystals (0.65 g, 97%). Mp 225–227 °C; IR (KBr) ν 3402, 3067, 3010, 2938, 2842, 1715, 1632, 1593, 1516, 1431, 1375, 1269, 1161, 1030, 893, 847, 820 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.72 (d, 1H, *J* = 16.0 Hz, CH=), 7.58 (d, 2H, *J* = 16.0 Hz, CH=), 6.69–7.10 (m, 9H, aromatic), 6.46 (d, 1H, *J* = 16.0 Hz, CH=), 6.15 (d, 2H, *J* = 16.0 Hz, CH=), 5.90–5.95 (m, 3H, ArOH), 5.68 (d, 1H, *J* = 1.2 Hz, CH), 5.63 (t, 2H, *J* = 4.0 Hz, H-4 and -6), 5.54 (m, 1H, H-2), 4.88 (m, 1H, H-5), 5.53 (m, 2H, H-1 and -3), 3.91(s, 3H, OCH_3), 3.65 (s, 6H, OCH_3) ppm. Anal. calcd for $C_{37}H_{34}O_{15}$: C, 61.84; H, 4.77; found: C, 61.40; H, 4.71.

2-*O*-*tert*-Butyldimethylsilyl-4,6-bis-*O*-[3-(4′-acetoxy-3′-methoxyphenyl)-2-propenoyl]-*myo*-inositol 1,3,5-orthoformate (8). To a solution of **4** (1.20 g, 3.9 mmol) in anhydrous pyridine (30 mL) were added a catalytic amount of DMAP and acid chloride **5** (3.01 g, 11.8 mmol). The mixture was stirred overnight at room temperature and the reaction was quenched by adding water. The mixture was dissolved in 80 mL EtOAc and washed successively with saturated aqueous $KHSO_4$, saturated aqueous $NaHCO_3$, and brine. The organic layer was dried over anhydrous Na_2SO_4. The solvent was evaporated and the residue was chromatographed (silica gel, 1:2 EtOAc/(hexane) to give **8** (2.58 g, 88%). The product **8** was obtained as colorless prisms by recrystallization from acetone/*n*-hexane. Mp 148–150 °C; IR (KBr) ν 3119, 3065, 2958, 2858, 1763, 1716, 1635, 1601, 1508, 1419, 1371, 1163, 1032, 1006, 987, 895 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.60 (d, 1H, J = 16.0 Hz, CH=), 6.78–6.93 (m, 6H, aromatic), 6.22 (d, 1H, J = 16.0 Hz, CH=), 5.62 (d, 1H, J = 1.2 Hz, CH), 5.59 (t, 2H, J = 4.0 Hz, H-4 and -6), 4.76 (m, 1H, H-2), 4.29 (m, 3H, H-1, -3 and -5), 3.69 (s, 6H, OCH_3), 2.30 (s, 6H, acetyl), 0.95 (s, 9H, CH_3), 0.16 (s, 6H, $SiCH_3$) ppm. Anal. calcd for $C_{37}H_{44}O_{14}Si$: C, 59.99; H, 5.99; found: C, 59.96; H, 5.98.

2-*O*-*tert*-Butyldimethylsilyl-4,6-bis-*O*-[3-(4′-hydroxy-3′-methoxyphenyl)-2-propenoyl]-*myo*-inositol 1,3,5-orthoformate(9). The compound **8** (0.30 g, 0.41 mmol) was dissolved in a mixture of $CHCl_3$ (7 mL) and MeOH (3 mL) and treated with $NH_2NH_2{\cdot}H_2O$ (43 μL, 0.9 mmol). After being stirred at room temperature for 3 h, the reaction mixture was diluted with $CHCl_3$ and successively washed with saturated aqueous $KHSO_4$, saturated aqueous $NaHCO_3$, and brine. The organic layer was dried over anhydrous Na_2SO_4. The solvent was evaporated and the residue was recrystallized from ethanol to provide **9** as a white solid (0.25 g, 93%). Mp 210–212 °C; IR (KBr) ν 3450, 3072, 2949, 2856, 1715, 1630, 1516, 1466, 1431, 1250, 1165, 1000, 955, 881, 818 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.54 (d, 1H, J = 16.0 Hz, CH=), 6.69–6.79 (m, 6H, aromatic), 6.09 (d, 1H, J = 16.0 Hz, CH=), 5.87 (s, 2H, ArOH), 5.62 (d, 1H, J = 1.2 Hz, CH), 5.57 (t, 2H, J = 4.0 Hz, H-4 and -6), 4.76 (m, 1H, H-2), 4.35 (m, 1H, H-5), 4.29 (m, 2H, H-1 and -3), 3.67 (s, 6H, OCH_3), 0.96 (s, 9H, CH_3), 0.18 (s, 6H, $SiCH_3$) ppm. Anal. calcd for $C_{33}H_{40}O_{12}Si$: C, 60.35; H, 6.14; found: C, 60.28; H, 6.10.

4,6-Bis-*O*-[3-(4′-hydroxy-3′-methoxyphenyl)-2-propenoyl]-*myo*-inositol 1,3,5-orthoformate (10). The compound **9** (70 mg, 0.1 mmol) was dissolved in THF (5 mL) and treated with n-$Bu_4NF{\cdot}3H_2O$ (109.3 mg, 0.35 mmol). After being stirred at room temperature for 3 h, the reaction mixture was diluted with $CHCl_3$ and successively washed with saturated aqueous $KHSO_4$, saturated aqueous $NaHCO_3$, and brine. The organic layer was dried over anhydrous Na_2SO_4. The solvent was evaporated and the residue was recrystallized from THF/MeOH to provide **10** as a white solid (55.8 mg, 98%). Mp 248–250 °C; IR (KBr) ν 3481, 3340, 3069, 2958, 2843, 1728, 1709, 1634, 1601, 1514, 1433, 1281, 1192, 991, 953, 891 cm^{-1}; ^1H NMR (THF-d_8) δ 8.39 (s, 2H, ArOH), 7.62 (d, 2H, J = 16.0 Hz, CH=), 6.60–7.05 (m, 6H, aromatic), 6.25 (d, 2H, J = 16.0 Hz, CH=), 5.53 (d, 1H, J = 1.2 Hz, CH), 5.50 (t, 2H, J = 4.0 Hz, H-4 and -6), 4.97 (d, 1H, J = 10.4 Hz, OH), 4.65 (m, 1H, H-5), 4.21 (m, 2H, H-1 and -3), 4.07(m, 1H, H-2), 3.68 (s, 6H, OCH_3) ppm. Anal. calcd for $C_{27}H_{26}O_{12}$: C, 59.78; H, 4.83; found: C, 59.66; H, 4.85.

2-*O*-*tert*-Butyldimethylsilyl-4-*O*-[3-(4′-acetoxy-3′-methoxyphenyl)-2-propenoyl]-*myo*-inositol 1,3,5-orthoformate (11). A mixture of a catalytic amount of 4-dimethylaminopyridine and **5** (0.63 g, 2.46 mmol) was added to a solution of **4** (0.5 mg, 1.60 mmol) in anhydrous pyridine (30 mL). The mixture was stirred at room temperature overnight and the reaction was quenched by adding water. Ethyl acetate was added and the mixture was washed successively with saturated aqueous $KHSO_4$, saturated aqueous $NaHCO_3$ and brine. After the organic layer was dried over $MgSO_4$, the solvent was removed under reduced pressure. The residue was chromatographed (silica gel, *n*-hexane/ethyl acetate, 3:2) to give **11** as a white solid (0.252 g, 30%). Mp 169–171 °C; IR (KBr) ν 3450, 2963, 2858, 1757, 1705, 1632, 1601, 1512, 1468, 1420, 1373, 1244, 1165, 1009, 961, 864 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.65 (d, 1H, J = 16.0 Hz, CH=), 7.04–7.12 (m, 3H, aromatic), 6.29 (d, 1H, J = 16.0 Hz, CH=), 5.63 (m, 1H, H-4), 5.55 (d, J = 1.2 Hz, CH), 4.59 (m, 1H, H-5), 4.42 (m, 1H, H-2), 4.24 (m, 2H, H-1 and -3), 4.15 (m, 1H, H-6), 3.86 (s, 3H, OCH_3), 2.31 (s, 3H, acetyl), 0.93 (s, 9H, CH_3), 0.14 (s, 6H, $SiCH_3$) ppm. Anal. calcd for $C_{25}H_{34}O_{10}Si$: C, 57.45; H, 6.56; found: C, 57.66; H, 6.58.

2-*O*-*tert*-Butyldimethylsilyl-4-*O*-[3-(4′-hydroxy-3′-methoxyphenyl)-2-propenoyl]-*myo*-inositol 1,3,5-orthoformate (12). The compound **11** (81.7 mg, 0.156 mmol) was dissolved in a mixture of $CHCl_3$ (3 mL) and MeOH (2 mL) and treated with $NH_2NH_2{\cdot}H_2O$ (10 μL, 0.2 mmol). After being stirred at room temperature for 24 h, the reaction mixture was diluted with $CHCl_3$ and successively washed with saturated aqueous $KHSO_4$, saturated aqueous $NaHCO_3$, and brine. The organic layer was dried over anhydrous Na_2SO_4. The solvent was evaporated and the residue was recrystallized from EtOAc/*n*-hexane to provide **12** as colorless prisms (66 mg, 88%). Mp 154–156 °C; IR (KBr) ν 3450, 3300, 2968, 2932, 2858, 1668, 1608, 1514, 1435, 1267, 1161, 1062, 1010, 976, 947, 851 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.62 (d, 1H, J = 16.0 Hz, CH=), 6.89–7.07 (m, 3H, aromatic), 6.18 (d, 1H, J = 16.0 Hz, CH=), 5.98 (s, 1H, ArOH), 5.62 (m, 1H, H-4), 5.55 (d, 1H, J = 1.2 Hz, CH), 4.57 (m, 1H, H-5), 4.41 (m, 1H, H-2), 4.24 (m, 2H, H-1 and -3), 4.16 (m, 1H, H-6), 3.92 (s, 3H, OCH_3), 2.54 (d, 1H, J = 7.2 Hz, OH), 0.92 (s, 9H, CH_3), 0.13 (s, 6H, $SiCH_3$) ppm. Anal. calcd for $C_{23}H_{32}O_9Si$: C, 57.48; H, 6.71; found: C, 57.55; H, 6.77.

2,4,6-Tris-*O*-[3-(4′-hydroxy-3′-methoxyphenyl)-2-propenoyl]-*myo*-inositol (13). A suspension of **7** (66.7 mg, 0.1 mmol) in 80% CF_3COOH (10 mL) was stirred for 24 h at room temperature. The solvent was removed under reduced pressure. The residue was chromatographed on preparative TLC (0.5 mm thickness of silica

gel, ethyl acetate) to give **13** (22.7 mg, 43%) as a white solids. MP 151–154 °C; IR (KBr) ν 3300, 2980, 2841, 1697, 1630, 1597, 1516, 1427, 1267, 1168, 1057, 1028, 982, 849, 818 cm^{-1}; ^1H NMR (CD_3OD) δ 7.69 (d, 1H, J = 16.0 Hz, CH=), 7.64 (d, 2H, J = 16.0 Hz, CH=), 6.77–7.25 (m, 9H, aromatic), 6.54 (d, 1H, J = 16.0 Hz, CH=), 6.42 (d, 2H, J = 16.0 Hz, CH=), 5.68 (t, 1H, J = 3.2 Hz, H-2), 5.45 (t, 2H, J = 10.0 Hz, H-4 and -6), 3.98 (dd, 2H, J = 3.2, 10.0 Hz, H-1 and -3), 3.91(s, 3H, OCH_3), 3.85 (s, 6H, OCH_3) 3.78 (t, 1H, J = 10.0 Hz, H-5) ppm. Anal. calcd for $C_{36}H_{36}O_{15}$: C, 61.01; H, 5.12; Found: C, 61.12; H, 5.30.

4,6-Bis-*O*-[3-(4′-hydroxy-3′-methoxyphenyl)-2-propenoyl]-*myo*-inositol (14). A suspension of **9** (66.7 mg, 0.1 mmol) in 80% CF_3COOH (10 mL) was stirred for 24 h at room temperature. The solvent was removed under reduced pressure. The residue was chromatographed on preparative TLC (0.5 mm thickness of silica gel, ethyl acetate) to give **14** (22.7 mg, 43%) as a white solid. Mp 191–194 °C; IR (KBr) ν 3595, 3452, 3398, 3234, 3003, 2947, 2914, 1713, 1630, 1600, 1522, 1427, 1180, 1080, 1014, 974, 922, 860, 820 cm^{-1}; ^1H NMR (acetone-d_6 + D_2O) δ 7.53 (d, 2H, J = 16.0 Hz, CH=), 6.75–7.15 (m, 6H, aromatic), 6.33 (d, 2H, J = 16.0 Hz, CH=), 5.28 (t, 2H, J = 9.6 Hz, H-4 and 6), 4.06 (t, 1H, J = 2.8 Hz, H-2), 3.78 (s, 6H, OCH_3), 3.62–3.72 (m, 3H, H-1, 3 and 5). Anal. calcd for $C_{26}H_{28}O_{12}$: C, 58.64; H, 5.30; found: C, 58.55; H, 5.20.

Single crystal X-ray diffraction for 8

The crystal mounted on a glass fiber. The single crystal X-ray data was collected on a Rigaku R-AXIS RAPID Imaging plate diffractometer. All calculations were performed with the crystallographic software package teXsan (Molecular Structure Corporation, 1985 and 1999). The structure was solved by direct method (SHELXS-97)[30] and expanded using Fourier techniques (DIRDIF94).[31] Non-hydrogen atoms were refined anisotropically. Hydrogen atoms except OH groups were included at calculated positions: Crystal data for **8** ($C_{37}H_{44}O_{14}Si$): M = 740.83, triclinic, space group P-1 (#2), μ(Cu K_α) = 10.9 cm^{-1} radiation; T = −40 °C, a = 12.414(5) Å b = 16.861(9) Å, c = 10.193(5) Å, α = 107.102(2), β = 90.297(2), γ = 107.053 (2) V = 1939.6(2) $Å^3$, Z = 2, D. = 1.268 g/cm^3, 11,673 reflections measured, 6507 unique (R_{int} = 0.031). Full-matrix least-squares refinement was based on 5134 observed reflections [I > 2.00σ(I)] and 470 variable parameters. R = 0.081, R_w = 0.180, GOF = 1.90. Crystallographic data (excluding structure factors) for the structure in this paper have been deposited with the Cambridge Crystallographic Data Center as supplementary publication numbers CCDC 171002. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [fax: +44-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].

Biological evaluation

Reporter gene assay for COX-2 promoter activity. The reporter gene assay system that the *Lac*Z gene was inserted into the downstream of COX-2 promoter region was used to estimate COX-2 promoter activity in human colon cancer cells.[13,25] Designed DLD-1/COX2-B2-β-Gal-BSD cells and DLD-1/B2-β-Gal-BSD cells were constructed as previously reported.[25] Cells were maintained in RPMI-1640 medium containing 5% heat-inactivated fetal bovine serum (Gibco BRL, Grand Island, NY, USA) at 37 °C in 5% CO_2. The subcloned DLD-1/COX2-B2-β-Gal-BSD cells were inoculated 2×10^4 cells/well in 96-well microtiter plates and precultured for 24 h. The cells were then treated with test compounds for 24 h. The β-galactosidase activity of DLD-1 cells in each well was determined by colorimetric assay using *o*-nitrophenyl-β-D-galacto-pyranoside. The β-galactosidase activity of DLD-1/COX2-B2-β-Gal-BSD cells was expressed by the measurement of absorbance at 405/630 nm. The values of β-galactosidase activity were normalized for viable cell number which was assessed by the MTT assay. All experiments were repeated three times with nearly identical results. Data are expressed as means ±SD (n = 3).

MTT assay. Cell viability in each culture was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. After treatment, the cells were further incubated in a medium containing 0.5 mg/mL of MTT for 1 h. The MTT formazan produced by living cells was dissolved in dimetyl sulfoxide (DMSO) and absorbance at 570 nm was measured on a microplate Reader (Spectra & Rainbow Readers, TECAN).

Acknowledgements

This study was performed through support from the Special Coordination Funds for Promoting Science and Technology (Leading Research Utilizing Potential of Regional Science and Technology) of the Ministry of Education, Culture, Sports, Science and Technology of the Japanese Government.

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