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Nobiletin metabolites: Synthesis and inhibitory activity against matrix metalloproteinase-9 production

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ABSTRACT

A divergent synthesis of nobiletin metabolites was developed through highly oxygenated acetophenone derivative. We used commercially available methyl 3,4,5-trimethoxybenzoate as a starting material for concise preparation of the key intermediate, 2′-hydroxy-3′,4′,5′,6′-tetramethoxyacetophenone (**I**). These metabolites showed strong inhibitory activity against matrix metalloproteinase-9 production in human lens epithelial cells.

Nobiletin (3′,4′,5,6,7,8-hexamethoxyflavone) (**1**), isolated from citrus fruits, has a broad spectrum of health-promoting properties including anticancer,[1] antimetastatic,[2] antiinflammatory,[3] antidiabetic,[4] and neurotrophic activities.[5] In particular, increasing attention has been paid to its antitumor metastatic activity due to the inhibition of gene expression and production of some matrix metalloproteinases (MMP-1, -3, and -9).[6] Recently, nobiletin metabolites have been found to possess potent antiinflammatory,[7] antitumor,[8] and neurotrophic activities,[9] which in some cases exceed those of the parent compound.

In the course of metabolic studies of **1**, 3′-demethylnobiletin (**2**),[10] 4′-demethylnobiletin (**3**),[11] and 3′,4′-didemethylnobiletin (**4**)[12] have been isolated from rodent urine and identified (Fig. 1). Because of the limited availability of these metabolites in nature, detailed investigations of the structure–activity relationship remain to be performed. We therefore initiated a divergent synthesis of nobiletin metabolites **2**–**4** to confirm their structures unambiguously. In the present study, we developed a versatile method for the synthesis of nobiletin metabolites and evaluated their inhibitory activity against MMP-9 production.

Syntheses of nobiletin and its derivatives reported thus far can be classified into two categories as depicted in Scheme 1, that is, (A) intramolecular Michael cyclization of 2′-hydroxychalcone followed by oxidative dehydrogenation;[9,13–16] and (B) C-ring construction via dehydration of 1-(2-hydroxyaryl)-3-arylpropane-1,3-dione.[17–21] Both approaches rely on 2′-hydroxyacetophenone **I** as a key compound. However, efficient synthesis of such a highly oxygenated acetophenone derivative has not been reported. With this context in mind, we commenced with the development of an alternative access to key compound **I**.

We chose commercially available permethyl gallate (**5**) as a starting material (Scheme 2). Our efforts concentrated on methoxylation of the 2,6-positions of **5** using the Ulmann-type reaction. Upon treatment with 2 equiv of NBS, ester **5** was brominated in quantitative yield. The resulting **6** was subjected to the CuBr-mediated Ulmann-type reaction with a large excess amount of sodium methoxide,[22] which gave rise to 2,6-dihydroxybenzoate (**7**) instead of the anticipated permethoxylated ester (**8**).[23]

Based on the result, we proposed a reaction mechanism including catalytic cycles of Cu salt: (i) oxidative addition; and (ii) reductive elimination (Scheme 3). In this cycle, we recognized that intermediate A, in which Cu(I) salt coordinates with the carboxyl group and methoxy group, could be prepared for demethylation to afford **7**. Thus, we assumed that this demethylation process is peculiar to the benzoate derivative.

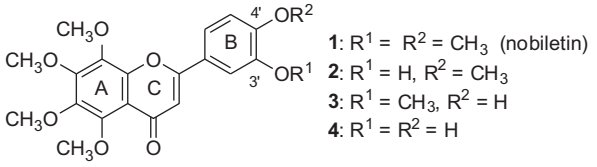

Figure 1. Structures of nobiletin and its metabolites.

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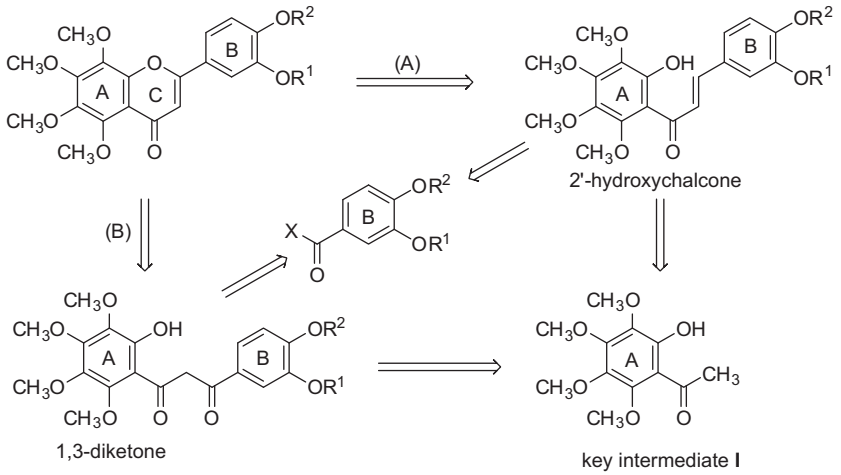
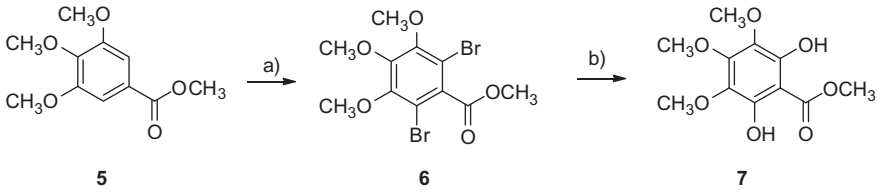
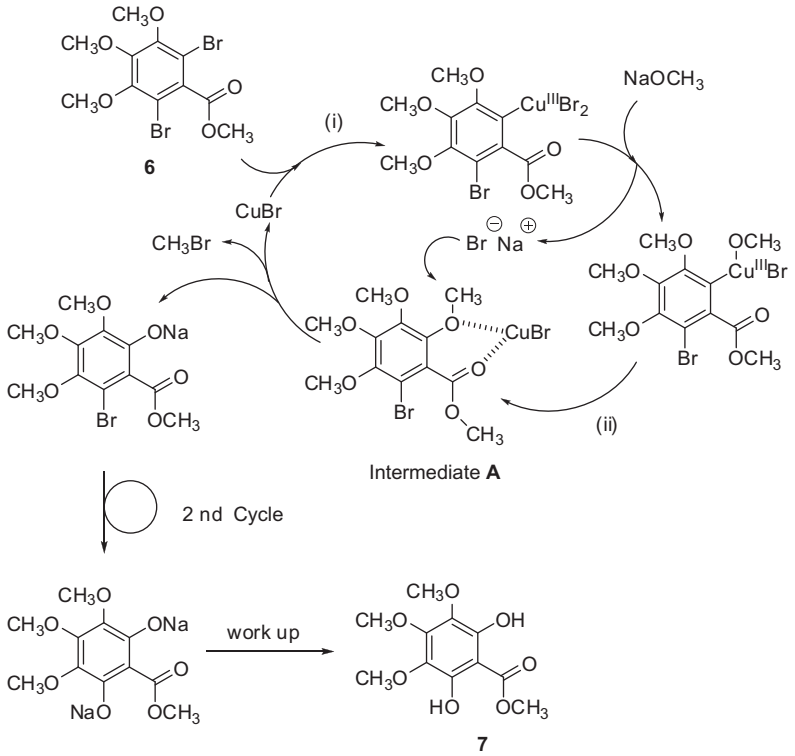


Scheme 1. Classification of polymethoxyflavone synthesis.

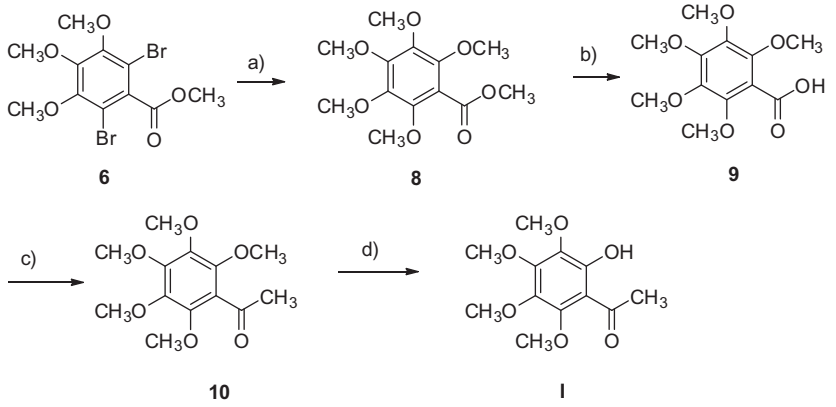


Scheme 2. Synthesis of 2,6-dihydroxybenzoate. Reagents and conditions: (a) NBS (2.4 equiv), DMF, 50 °C, quant.; (b) CuBr (0.1 equiv), NaOMe (20 equiv), methanol, reflux, 90%.

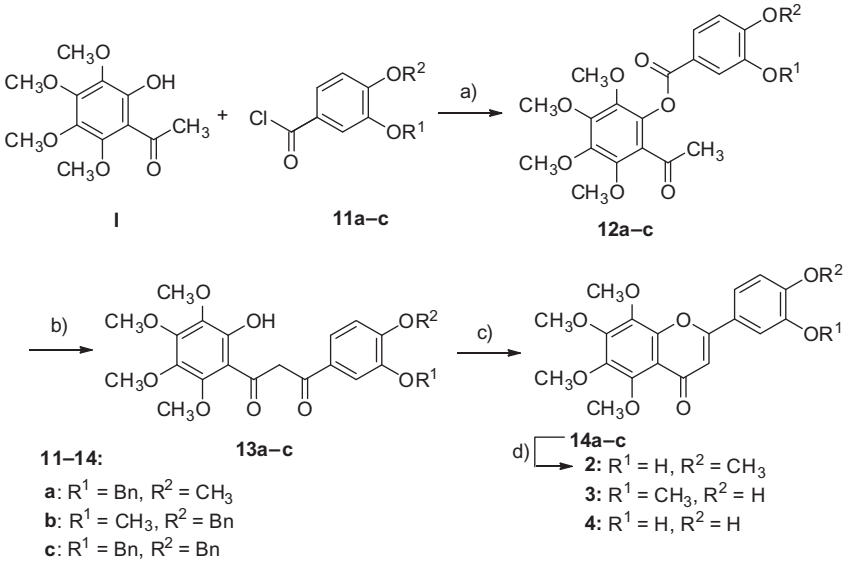


Scheme 3. Proposed reaction mechanism of Ulmann-type reaction.

Unfortunately, selective monoetherification of 2,6-dihydroxybenzoate **7** failed. We therefore examined subsequent permethylation of crude **7** to obtain **8** (Scheme 4). Alkaline hydrolysis of ester **8** provided carboxylic acid **9** in 96% yield in three steps.[24] To provide methyl ketone functionality, **9** was then treated with 2 equiv of methyllithium.[25] However, unexpected



Scheme 4. Synthesis of 2′-hydroxyacetophenone **I**. Reagents and conditions: (a) (i) CuBr (0.1 equiv), NaOMe (20 equiv), methanol, reflux; (ii) K_2CO_3 (5 equiv), CH_3I (7 equiv), DMF; (b) NaOH (3 equiv), ethanol/H_2O, 70 °C, 96% in three steps; (c) (i) $(COCl)_2$ (1.3 equiv), DMF (cat), CH_2Cl_2; (ii) $Fe(acac)_3$ (0.05 equiv), MeMgBr (1.1 equiv), THF, 0 °C to rt, 74% in two steps; (d) BCl_3 (1.1 equiv), CH_2Cl_2, −78 °C, 1 h, −15 °C, 2 h, 96%.



Scheme 5. Synthesis of nobiletin metabolites **2**–**4**. Reagents and conditions: (a) Et_3N (1.1–1.6 equiv), CH_2Cl_2; **12a** 74%; **12b** 87%; **12c** 92%; (b) *t*-BuOK (1.1 equiv), THF, reflux, 1–1.5 h; **13a** 88%; **13b** 61%; **13c** 60%; (c) *p*-TsOH·H_2O (0.25 equiv), benzene, reflux (Dean–Stark), 5–10 h; **14a** 85%; **14b** 84%; **14c** 80%; (d) H_2 (1 atm), 20% $Pd(OH)_2$/C (cat), ethyl acetate/ethanol (1:1), 0.5–1 h; **2** 84%; **3** 88%; **4** 93%.

decarboxylation occurred, and **10** was not obtained. Therefore, we next examined the two-step conversion protocol. Treatment of **8** with oxalyl chloride led to the corresponding acid chloride, which was coupled with methylmagnesium bromide in the presence of tris(acetylacetonato)iron(III)[26] to give methyl ketone (**10**) in 74% yield in two steps. Demethylation with boron trichloride proceeded in a highly regioselective manner to afford the key intermediate **I**[27] in 96% yield (68% overall yield from permethyl gallate [**5**]).

With the requisite methyl ketone **I** in hand, we followed approach B in Scheme 1: the introduction of the B-ring moiety and the subsequent closure of the C ring. As shown in Scheme 5, acid chlorides **11a**–**c** were unambiguously prepared from isovanillin, vanillin, and 3,4-dihydroxybenzoic acid, respectively. Acylation of **I** proceeded, and the resulting aryl esters **12a**–**c** were subjected to a modified protocol of Baker–Venkataraman rearrangement,[28] which gave rise to 1,3-diketones **13a**–**c** in good yields, respectively. Acid-catalyzed dehydration constructed the flavone skeleton **14a**–**c** smoothly. Finally, catalytic hydrogenolysis of the benzyl ether with Pearlman's catalyst afforded nobiletin metabolites **2**–**4**,[29] for which the spectroscopic data were identical to those reported previously.[9,12a,13,15]

We next investigated the effects of synthesized metabolites **2**–**4** on the inhibition of the production of proMMP-9 in the PMA- or TNF-α-stimulated human lens epithelial cell line SRA01/04[30] (Table 1). It was revealed that compound **3** inhibits the expression of proMMP-9 much more potently than **1**, whereas compounds **2** and **4** possess activity comparable to that of **1**. Although we have limited information on the structure–activity relationship, the position of the hydroxyl group on the B ring of nobiletin may be closely linked with their inhibitory activity against proMMP-9 production.

In summary, we succeeded in developing a new synthetic method for nobiletin metabolites **2**–**4**, which involves easier access to the highly oxygenated A-ring moiety. The metabolites thus synthesized showed potent inhibitory activity against proMMP-9 production. Since MMP-9 expression plays an important role in various pathological states including cataract, rheumatoid arthritis,[7] tumor metastasis,[8] etc., these metabolites could be potent lead compounds for chemotherapeutic agents for the treatment of those

Table 1
IC_{50} values (μM) of nobiletin (**1**) and compounds **2**–**4** for proMMP-9 production[31]

Entry	Compd	PMA-treated cells	TNF-α-treated cells
1	**1**	20.9 ± 6.5	17.0 ± 1.6
2	**2**	16.9 ± 2.0	16.4 ± 6.6
3	**3**	3.7 ± 0.3	5.5 ± 0.8
4	**4**	12.3 ± 1.2	10.8 ± 1.9

diseases. Further synthesis and evaluation of nobiletin analogues are currently underway in our laboratory.

Acknowledgments

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References and notes

1. (a) Kandaswami, C.; Perkins, E.; Soloniuk, D. S.; Drzewiecki, G.; Middleton, E. *Cancer Lett.* **1991**, *56*, 147; (b) Sugiyama, S.; Umehara, K.; Kuroyanagi, M.; Ueno, A.; Taki, T. *Chem. Pharm. Bull.* **1993**, *41*, 714; (c) Manthey, J. A.; Guthrie, N. *J. Agric. Food Chem.* **2002**, *50*, 5837; (d) Ohnishi, H.; Asamoto, M.; Tujimura, K.; Hokaiwado, N.; Takahashi, S.; Ogawa, K.; Kuribayashi, M.; Ogiso, T.; Okuyama, H.; Shirai, T. *Cancer Sci.* **2004**, *95*, 936.
2. (a) Sato, T.; Koike, L.; Miyata, Y.; Hirata, M.; Mimaki, Y.; Sashida, Y.; Yano, M.; Ito, A. *Cancer Res.* **2002**, *62*, 1025; (b) Miyata, Y.; Sato, T.; Yano, M.; Ito, A. *Mol. Cancer Ther.* **2004**, *3*, 839; (c) Miyata, Y.; Sato, T.; Imada, K.; Dobashi, A.; Yano, M.; Ito, A. *Biochem. Biophys. Res. Commun.* **2008**, *366*, 168.
3. (a) Murakami, A.; Nakamura, Y.; Torikai, K.; Tanaka, T.; Koshiba, T.; Koshimizu, K.; Kuwahara, S.; Takahashi, Y.; Ogawa, K.; Yano, M.; Tokuda, H.; Nishino, H.; Mimaki, Y.; Sashida, Y.; Kitanaka, S.; Ohigashi, H. *Cancer Res.* **2000**, *60*, 5059; (b) Tanaka, S.; Sato, T.; Akimoto, N.; Yano, M.; Ito, A. *Biochem. Pharmacol.* **2004**, *68*, 433; (c) Imada, K.; Lin, N.; Liu, C.; Lu, A.; Chen, W.; Yano, M.; Sato, T.; Ito, A. *Biochem. Biophys. Res. Commun.* **2008**, *373*, 181.
4. (a) Saito, T.; Abe, D.; Sekiya, K. *Biochem. Biophys. Res. Commun.* **2007**, *357*, 371; (b) Kunimasa, K.; Kuranuki, S.; Matsuura, N.; Iwasaki, N.; Ikeda, M.; Ito, A.; Sashida, Y.; Mimaki, Y.; Yano, M.; Sato, M.; Igarashi, Y.; Oikawa, T. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2062; (c) Miyata, Y.; Tanaka, H.; Shimada, A.; Sato, T.; Ito, A.; Yamanouchi, T.; Kosano, H. *Life Sci.* **2011**, *88*, 613.
5. (a) Matsuzaki, K.; Miyazaki, K.; Sakai, S.; Yawo, H.; Nakata, N.; Moriguchi, S.; Fukunaga, K.; Yokosuka, A.; Sashida, Y.; Mimaki, Y.; Yamakuni, T.; Ohizumi, Y. *Eur. J. Pharmacol.* **2008**, *578*, 194; (b) Onozuka, H.; Nakajima, A.; Matsuzaki, K.; Shin, R.-W.; Ogino, K.; Saigusa, D.; Tetsu, N.; Yokosuka, A.; Sashida, Y.; Mimaki, Y.; Yamakuni, T.; Ohizumi, Y. *J. Pharmacol. Exp. Ther.* **2008**, *326*, 739; (c) Yamamoto, Y.; Shioda, N.; Han, F.; Moriguchi, S.; Nakajima, A.; Yokosuka, A.; Mimaki, Y.; Sashida, Y.; Yamakuni, T.; Ohizumi, Y.; Fukunaga, K. *Brain Res.* **2009**, *1295*, 218.
6. For reviews, see: (a) Dzwonek, J.; Rylski, M.; Kaczmarek, L. *FEBS Lett.* **2004**, *567*, 129; Nagase, H.; Visse, R.; Murphy, G. *Cardiovasc. Res.* **2006**, *69*, 562; (c) Murphy, G.; Nagase, H. *Mol. Aspects Med.* **2008**, *29*, 290.
7. Li, S.; Sang, S.; Pan, M.-H.; Lai, C.-S.; Lo, C.-Y.; Yang, C. S.; Ho, C.-T. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 5177.
8. Lai, C.-S.; Li, S.; Chai, C.-Y.; Lo, C.-Y.; Dushenkov, S.; Ho, C.-T.; Pan, M.-H.; Wang, Y.-J. *Carcinogenesis* **2008**, *29*, 2415.
9. Al Rahim, M.; Nakajima, A.; Saigusa, D.; Tetsu, N.; Mayumura, Y.; Shibuya, M.; Yamakoshi, H.; Tomioka, Y.; Iwabuchi, Y.; Ohizumi, Y.; Yamakuni, T. *Biochemistry* **2009**, *48*, 7713.
10. (a) Murakami, A.; Kuwahara, S.; Takahashi, Y.; Ito, C.; Furukawa, H.; Ju-ichi, M.; Koshimizu, K.; Ohigashi, H. *Biosci. Biotechnol. Biochem.* **2001**, *65*, 194; (b) Murakami, A.; Koshimizu, K.; Ohigashi, H.; Kuwahara, S.; Kuki, W.; Takahashi, Y.; Hosotani, K.; Kawahara, S.; Matsuoka, Y. *BioFactors* **2002**, *16*, 73.
11. Yasuda, T.; Yoshimura, Y.; Yabuki, H.; Nakazawa, T.; Ohsawa, K.; Mimaki, Y.; Sashida, Y. *Chem. Pharm. Bull.* **2003**, *51*, 1426.
12. (a) Li, S.; Wang, Z.; Sang, S.; Huang, M.-T.; Ho, C.-T. *Mol. Nutr. Food Res.* **2006**, *50*, 291; For a review: (b) Li, S.; Tan, D.; Dushenkov, S.; Ho, C.-T. *ACS Symp. Ser.* **2008**, *987*, 216 (Dietary Supplements); Inhibitory activity of **4** against lens aldose reductases was previously reported. See also: (c) Okuda, J.; Miwa, I.; Inagaki, K.; Horie, T.; Nakayama, M. *Chem. Pharm. Bull.* **1984**, *32*, 767.
13. Li, S.; Pan, M.-H.; Lai, C.-S.; Lo, C.-Y.; Dushenkov, S.; Ho, C.-T. *Bioorg. Med. Chem.* **2007**, *15*, 3381.
14. Oliverio, A.; Casinovi, C. *Gazz. Chim. Ital.* **1950**, *80*, 798.
15. Mizuno, M.; Matoba, Y.; Tanaka, T.; Tachibana, H.; Inuma, M.; Iwasa, M. *J. Nat. Prod.* **1987**, *50*, 751.
16. Chen, W. M. *Indian Heterocycl. Chem.* **1997**, *6*, 221.
17. Horii, Z. *Yakugaku Zasshi* **1940**, *60*, 614.
18. Krishnamurti, M.; Seshadri, T. R.; Sharma, N. D. *Indian J. Chem.* **1970**, *8*, 575.
19. Wagner, H.; Maurer, G.; Hörhammer, L.; Farkas, L. *Chem. Ber.* **1971**, *104*, 3357.
20. Tsukayama, M.; Kawamura, Y.; Ishizuka, T.; Hayashi, S.; Torii, F. *Heterocycles* **2003**, *60*, 2775.
21. Asakawa, T.; Hiza, A.; Nakayama, M.; Inai, M.; Oyama, D.; Koide, H.; Shimizu, K.; Wakimoto, T.; Harada, N.; Tsukada, H.; Oku, N.; Kan, T. *Chem. Commun.* **2011**, *47*, 2868.
22. (a) Aalten, H. L.; van Koten, G.; Grove, D. M.; Kuilman, T.; Piekstra, O. G.; Hulshof, L. A.; Sheldon, R. A. *Tetrahedron* **1989**, *45*, 5565; (b) Majetich, G.; Li, Y.; Zou, G. *Heterocycles* **2007**, *73*, 217.
23. For a similar example, see: Saito, S.; Gao, H.; Kawabata, J. *Helv. Chim. Acta* **2006**, *89*, 821.
24. For the catalyst, CuCl, $CuCl_2$, and CuI were found to work as well as CuBr to give the same 2,6-dihydroxybenzoate. On the other hand, no reaction occurred in the absence of the Cu catalyst. A mixture of **6** (1.00 g, 2.60 mmol), CuBr (37 mg, 0.26 mmol), and 28 wt % sodium methoxide solution in methanol (11 ml, 52 mmol) was placed into a 100-ml round-bottomed flask fitted with a reflux condenser. The reaction mixture was heated with vigorous stirring at reflux (bath temperature: 110 °C) for 3.5 h. Upon cooling to room temperature, the reaction mixture was carefully poured into 2 M HCl (40 mL). After the methanol evaporated, the aqueous layer was extracted with ethyl acetate (3 × 20 mL). Combined organic layers were washed with brine (2 × 50 mL), dried ($MgSO_4$), and concentrated in vacuo. The residue (700 mg) was dissolved in DMF (8 mL), to which were successively added K_2CO_3 (1.08 g, 7.81 mmol) and iodomethane (0.80 mL, 13 mmol). The reaction mixture was stirred at room temperature for 12 h before the addition of water (50 mL) and extracted with a mixture of toluene (20 mL) and ethyl acetate (20 mL). The extracts were washed with brine (2 × 50 mL), dried ($MgSO_4$), and concentrated in vacuo to afford crude **8** (830 mg), a small amount of which was purified on a silica gel column (hexane/EtOAc = 10/1–8/1) to give colorless solids: mp 48–49 °C; IR (KBr) 2944, 1740 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 3.96 (3H, s), 3.92 (3H, s), 3.87 (12H, s); ^{13}C NMR ($CDCl_3$) δ: 165.7, 149.1, 146.2 (2C), 142.9 (2C), 118.4, 61.8 (2C), 61.4, 61.2 (2C), 52.4; HRMS calcd. for $[M+H]^+$ of $C_{13}H_{18}O_7$: 287.1125; found: 287.1112. A mixture of crude **8** (825 mg), NaOH (0.312 g, 7.8 mmol), ethanol (8 mL), and water (2 mL) was heated at 70 °C for 10 h and cooled to room temperature. After the ethanol was evaporated, the residue was partitioned between toluene (20 mL) and water (30 mL). After the organic layer was discarded, the aqueous layer was acidified with 2 M HCl (20 mL) and extracted with chloroform (2 × 20 mL). The combined organic layers were washed with brine (30 mL), dried ($MgSO_4$), and concentrated in vacuo to afford a crude off-white solid (800 mg). Recrystallization (twice) from hot hexane/toluene (1:1) gave **9** as colorless prisms (679 mg, 2.49 mmol, 96% yield from **6**): mp 94–96 °C; IR (KBr) 2990, 1706 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 3.99 (3H, s), 3.94 (6H, s), 3.89 (6H, s); ^{13}C NMR ($CDCl_3$) δ: 169.2, 149.9, 147.1 (2C), 143.1 (2C), 116.7, 62.2 (2C), 61.5, 61.3 (2C). HRMS calcd. for $[M+H]^+$ of $C_{12}H_{16}O_7$: 273.0969. Found: 273.0960.
25. Gilman, H.; van Ess, P. R. *J. Am. Chem. Soc.* **1933**, *55*, 1258.
26. Fiandanese, V.; Marchese, G.; Martina, V.; Ronzini, L. *Tetrahedron Lett.* **1984**, *25*, 4805.
27. *Intermediate* ***1***: obtained as a yellow oil. IR (neat) 2938, 1622, 1589 cm^{-1}; ^{-1}H NMR ($CDCl_3$) δ: 13.17 (1H, s), 4.09 (3H, s), 3.96 (3H, s), 3.86 (3H, s), 3.81 (3H, s), 2.68 (3H, s); ^{13}C NMR ($CDCl_3$) δ: 203.9, 154.3 (2C), 153.5, 151.2, 137.8, 136.6, 110.3, 61.2, 61.1, 61.0, 60.9, 32.2: HRMS calcd. for $[M+H]^+$ of $C_{12}H_{16}O_6$: 257.1020. Found: 257.1006.
28. Ares, J. J.; Outt, P. E.; Kakodkar, S. V.; Buss, R. C.; Geiger, J. C. *J. Org. Chem.* **1993**, *58*, 7903.
29. *Compound* ***2***: mp 170–171 °C; IR (KBr) 3309, 2937, 1638 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 7.51 (1H, d, J = 2.2 Hz), 7.47 (1H, dd, J = 2.2 Hz, 8.5 Hz), 6.96 (1H, d, J = 8.5 Hz), 6.61 (1H, s), 6.17 (1H, br s), 4.10 (3H, s), 4.02 (3H, s), 3.97 (3H, s), 3.95 (6H, s); ^{13}C NMR ($CDCl_3$) δ: 177.2, 161.0, 151.3, 149.3, 148.2, 147.6, 146.0, 143.9, 138.0, 124.6, 118.7, 114.8, 112.2, 110.7, 106.9, 62.2, 62.0, 61.8, 61.6, 56.1. HRMS calcd. for $[M+H]^+$ of $C_{20}H_{20}O_8$: 389.1231. Found: 389.1221. *Compound* ***3***: mp 153–154 °C; IR (KBr) 3250, 2935, 1625 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 7.53 (1H, dd, J = 2.0 Hz, 8.3 Hz), 7.39 (1H, d, J = 2.0 Hz), 7.05 (1H, d, J = 8.3 Hz), 6.61 (1H, s), 6.07 (1H, br s), 4.11 (3H, s), 4.03 (3H, s), 3.99 (3H, s), 3.96 (6H, s); ^{13}C NMR ($CDCl_3$) δ: 177.2, 161.0, 151.3, 148.8, 148.3, 147.4, 146.8, 144.0, 138.0, 123.5, 120.2, 114.9, 114.7, 108.1, 106.7, 62.3, 62.0, 61.8, 61.7, 56.1; HRMS calcd. for $[M-H]^-$ of $C_{20}H_{20}O_8$: 387.1085. Found: 387.1084. *Compound* ***4***: mp 209–211 °C; IR (KBr) 3379, 2941, 1631, 1605 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 7.74 (1H, d, J = 2.2 Hz), 7.51 (1H, dd, J = 2.2 Hz, 8.3 Hz), 7.03 (1H, d, J = 8.3 Hz), 6.74 (1H, s), 4.12 (3H, s), 4.03 (3H, s), 3.99 (3H, s), 3.96 (3H, s); ^{13}C NMR (DMSO-d_6): 175.7, 160.9, 150.8, 149.2, 147.5, 147.1, 145.7, 143.5, 137.7, 121.7, 118.4, 116.1, 114.3, 113.0, 105.4, 61.94, 61.90, 61.5, 61.4; HRMS calcd. for $[M-H]^-$ of $C_{19}H_{18}O_8$: 373.0929. Found: 373.0928.
30. Ibaraki, N.; Chen, S.-C.; Lin, L.-R.; Okamoto, H.; Pipas, J. M.; Reddy, V. N. *Exp. Eye Res.* **1998**, *67*, 577.
31. Experimental procedure in Table 1: The human lens epithelial cell line SRA01/04 was a kind gift from Dr. Nobuhiro Ibaraki (Jichi Medical University, Tochigi, Japan). The cells were cultured in Dulbecco's modified Eagle's medium (Invitrogen, Carlsbad, CA) supplemented with 20% (v/v) heat-inactivated (56 °C for 30 min) fetal bovine serum (Biowest, Nuaille, France) including PSN antibiotic mixture (Invitrogen, penicillin/streptomycin/neomycin: 100 μg/mL each) at 37 °C in a humidified 5% CO_2 atmosphere. After reaching confluence, the cells were treated with the test sample in the presence of PMA (Sigma–Aldrich, 10 nM) or TNF-α (Sigma–Aldrich, 10 ng/mL) for 24 h. The harvested culture media were stored at 4 °C until just before use. Aliquots (20 μL) of the harvested culture media were subjected to SDS–PAGE with 10% acrylamide gel containing gelatin (0.6 mg/mL) (Difco Laboratories, Detroit, MI).

The gel was washed with 50 mM Tris–HCl (pH 7.5), 0.15 M NaCl, 10 mM $CaCl_2$, 1 μM $ZnCl_2$, and 0.1% Triton X-100, and then incubated in 50 mM Tris–HCl (pH 7.5), 0.15 M NaCl, 10 mM $CaCl_2$, and 1 μM $ZnCl_2$ at 37 °C. Thereafter, the gel was stained with 0.1% Coomassie Brilliant Blue R-250, and gelatinolytic activity was detected as unstained bands on a blue background. The IC_{50} values (means of triplicate measurements) shown in Table 1 were estimated from the relative amount of proMMP-9 (quantified by Image-J) at various concentrations (0.25, 1, 4, 16, or 64 μM) of the test samples.