Supporting Information

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SI Materials and Methods

General Experimental Procedures. Lyprinol was kindly provided by MacLab Australia. Naproxen and ethyl eicosapentaenoate were purchased from Wako Pure Chemical Industries and Tokyo Chemical Industry, respectively. ^{1}H and ^{13}C NMR spectra were obtained on a JEOL ECA-500 spectrometer at 500 and 125 MHz, respectively. Mass spectra were measured on a JEOL MStation JMS-700 spectrometer. THF and CH_2Cl_2 were distilled from sodium benzophenone ketyl and calcium hydride, respectively. Normal-phase chromatography was performed with silica gel columns (60N spherical, neutral, 40–50 μm; Kanto Chemical). GC-MS analysis was performed with a JEOL JMS-SUN200 mass spectrometer coupled to an Agilent 6890N gas chromatograph. GC was carried out with Agilent GC capillary column HP-5 (30 m × 0.25 mm i.d.) and the oven temperature was increased from 120 °C to 240 °C at 24 °C/min.

Isolation of Furan Fatty Acids from Lyprinol. Lyprinol is a lipid-rich fraction prepared by supercritical fluid [CO_2] extraction of the freeze-dried stabilized powder of the green-lipped mussel *Perna canaliculus*. The lipid extract of the green-lipped mussel (2.98 g) was dissolved in CH_2Cl_2 (80 mL). The resulting pale yellow solution was hydrogenated with Pd-C (80 mg) under a hydrogen atmosphere to transform the polyunsaturated fatty acids (PUFAs) to saturated fatty acids. After 3 h of stirring, the catalyst was removed by filtration. To the colorless solution was added freshly prepared diazomethane in diethyl ether until the yellow color was retained. After removal of the excess diazomethane by bubbling nitrogen, the methylated solution was concentrated in vacuo and the residue was purified by silica gel flash chromatography eluted with hexane, hexane:diethyl ether (99:1), hexane:diethyl ether (98:2), and hexane:diethyl ether (97:3) to afford the fraction containing the F_4 methyl ester (0.8 mg/g; methyl-12,15-epoxy-13,14-dimethyloctadeca-12,14-dienoate) and the F_6 methyl ester (1.2 mg/g; methyl-12,15-epoxy-13,14-dimethyleicosa-12,14-dienoate) (Fig. S1). Alternatively, isolation from total fatty acids in Lyprinol was performed after methanolysis with sodium methoxide as described in the following methods.

Methods for the Preparation of Furan Fatty Acids. To perform quantitative analysis and an anti-inflammatory test of the furan fatty acids, we developed two different methods for the preparation of furan fatty acids: (*i*) isolation from salmon testicle to yield the standard materials for GC-MS analysis and (*ii*) semisynthesis from shark metabolite to afford the internal standard for the GC-MS analysis and the materials for the anti-inflammatory test.

Isolation of Furan Fatty Acids from Salmon Testicle. Salmon (*Oncorhynchus keta*) were collected from the Yambetsu River in Hokkaido, Japan, in late September 2007. Freeze-dried testes (5 g) were dissolved in $CHCl_3$ (10 mL) and MeOH (10 mL). To the solution was added freshly prepared sodium methoxide (1.0 M, 10 mL). After stirring for 20 min, the mixture was treated with 1.0 M HCl (100 mL) and extracted with $CHCl_3$. The organic layer was washed with brine and dried over Na_2SO_4. The resulting solution was further methylated by treatment with a diethyl ether solution of CH_2N_2 until all of the free fatty acids were completely methylated. After removal of the excess diazomethane by bubbling nitrogen, the resulting solution was concentrated in vacuo and purified by silica gel flash chromatography, and eluted with hexane, hexane:diethyl ether (99:1), hexane:diethyl ether (98:2), and hexane:diethyl ether (97:3) to afford the fractions containing furan fatty acids (F-acids). To remove the concomitant unsaturated fatty acid methyl esters, the fraction was hydrogenated with Pd-C (50 mg) under a hydrogen atmosphere for 5 min and then purified again by silica gel flash chromatography to yield the fractions containing both the F_4 and F_6 methyl esters (12 mg) (1–6).

Semisynthesis of Furan Fatty Acids: F_6 Ethyl Ester. Bile was collected from sharks (*Lamna ditropis*) caught for commercial supply at Kesennuma in Miyagi Prefecture, Japan, in 2005. The lyophilized material (1.6 kg) was dissolved in methanol and treated with H_2SO_4. The resulting solution was neutralized with solid $NaHCO_3$ and centrifuged (600 × *g*). The supernatant was concentrated in vacuo and partitioned between 1 M HCl and diethyl ether. To the ethereal solution (50 mL) was added H_2O (100 mL), $NaIO_4$ (36 mg), and OsO_4 (10 mL, 10 mg/mL in water). After stirring at room temperature for 2 d, the ethereal solution was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The resulting brown oil was purified by silica gel flash chromatography (hexane:AcOEt, 9:1) to yield the aldehyde (8.18 g). This aldehyde was immediately dissolved in CH_2Cl_2, because it would easily decompose in the neat oil state. To a solution of *n*-butyltriphenylphosphonium bromide, sodium bis(trimethylsilyl)amide (NaHMDS, 1.0 M THF solution) was added dropwise at −78 °C. To the yellow solution of *n*-butyltriphenylphosphonium ylide, the CH_2Cl_2 solution of the aldehyde was added dropwise at −78 °C. The reaction mixture was stirred for 3 h at −78 °C, warmed to room temperature, and then concentrated in vacuo. The resulting oil was purified by silica gel flash chromatography (hexane:diethyl ether, 98:2) with a celite pad to yield the *cis*-olefin. The CH_2Cl_2 solution of the *cis*-olefin was treated with Pd-C and stirred under a hydrogen atmosphere for 5 min. After the catalyst was removed by filtration through a celite pad, the mixture was purified by silica gel flash chromatography (hexane:diethyl ether, 98:2) to yield F-acid **2** (804 mg, 2.9 mmol). Subsequently, to a CH_2Cl_2 solution of F-acid **2**, diisobutylaluminum hydride (DIBAL) (1.0 M hexane solution) was added dropwise at −78 °C. The mixture was stirred for 2 h at −78 °C, warmed to 0 °C, and quenched with Rochelle salt. The biphasic solution was extracted with CH_2Cl_2, which was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The crude aldehyde was used for the next step without further purification. To a CH_2Cl_2 solution of 6-ethoxy-6-oxohexyltriphenylphosphonium bromide, NaHMDS was added dropwise, followed by the solution of the aldehyde at −78 °C. After stirring for 2 h at −78 °C to room temperature, the mixture was concentrated in vacuo. The resulting oil was purified by silica gel flash chromatography (hexane:diethyl ether, 98:2) to yield the olefinic F-acid. To a CH_2Cl_2 solution of the olefinic F-acid, Pd-C was added, and the mixture was stirred under a hydrogen atmosphere for 5 min. After removal of the catalyst, the solution was concentrated in vacuo. The resulting oil was purified by silica gel flash chromatography (hexane:diethyl ether, 98:2) to afford the F-acid (F_6) ethyl ester **3** (103 mg, 0.27 mmol) (Fig. S2).

(*E*)-5-(5-(2-Carboxyvinyl)-3,4-Dimethylfuran-2-Yl)Pentanoic Acid (1). FAB-MS *m/z* = 265 $(M\text{-}H)^-$, ^{1}H-NMR (500 MHz, CD_3OD) δ_H: 7.41 (1H, d, J = 15 Hz), 6.02 (1H, d, J = 15 Hz), 2.62 (2H, t), 2.29 (2H, t), 2.03 (3H, s), 1.89 (3H, s), 1.63 (4H, m). ^{13}C-NMR (125 MHz, CD_3OD) δ_C: 176.3, 170.2, 154.0, 144.6, 129.4, 127.9, 117.9, 111.4, 33.4, 27.5, 25.4, 24.3, 7.4, 6.7.

Methyl 5-(3,4-Dimethyl-5-Pentylfuran-2-Yl)Pentanoate (2). EI-MS m/z = 280 $(M)^+$, ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H: 3.65 (3H, s), 2.50 (2H, t), 2.46 (2H, t), 2.31 (2H, t), 1.82 (6H, s), 1.64 (2H, m), 1.62 (2H, m), 1.54 (2H, m), 1.28 (2H, m), 1.26 (2H, m), 0.87 (3H, t). ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C: 174.2, 148.7, 147.6, 114.9, 113.9, 51.9, 33.9, 29.7, 28.5, 28.3, 26.1, 25.7, 24.5, 22.5, 14.1, 8.4.

Ethyl 11-(3,4-Dimethyl-5-Pentylfuran-2-Yl)Undecanoate (3). EI-MS m/z = 378 $(M)^+$, ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H: 4.11 (2H, q), 2.49 (4H, t), 2.29 (2H, t), 1.82 (6H, s), 1.62 (6H, m), 1.22–1.33 (19H, m), 0.88 (3H, t). ^{13}C-NMR (125 MHz, $CDCl_3$) δ_C: 173.9, 149.4, 148.4, 114.5, 113.8, 60.2, 34.4, 31.7, 31.5, 29.6, 29.4, 29.3, 29.2, 28.9, 28.6, 26.1, 25.0, 22.5, 14.1, 8.4.

Quantitative Analysis of Furan Fatty Acids. Lipid extract of the New Zealand green shell mussel (Lyprinol; 1.0 g) was dissolved in MeOH:$CHCl_3$ (1:1, 20 mL). To estimate the recovery rate during the subsequent separation steps, compound **2** (1 mg) was added to the extract as an internal standard, because this compound had not been detected in the natural source. The mixture was then treated with freshly prepared 1.0 M sodium methoxide (10 mL). The resulting solution was partitioned between 1.0 M HCl and $CHCl_3$. The organic layer was washed with saturated aqueous NaCl, dried over Na_2SO_4, and filtered to yield a pale yellow solution. Subsequently, the remaining free fatty acids were completely methylated by the addition of an ethereal solution of CH_2N_2. After concentration in vacuo, the resulting brown oil was immediately purified by silica gel flash chromatography (3.0 × 10 cm) eluted with hexane, hexane:diethyl ether (99:1), hexane:diethyl ether (98:2), and hexane:diethyl ether (97:3). This solvent system separated the saturated and unsaturated fatty acid methyl esters. The furan fatty acid methyl esters were eluted with the unsaturated fatty acid methyl ester fractions, which were combined and subjected to GC-MS analysis (Table S1). The peak areas of ion peaks derived from the total-ion chromatogram were subjected to quantification.

Assay for Arthritis Inhibition. Arthritis was induced in rats (Lewis, female, 6-wk-old; Charles River Japan) by the injection of heat-killed *Mycobacterium tuberculosis* (Difco) suspended in squalene (1.1 mg per rat) into the right hind paw. The increase in paw volume was measured every day during the experiment using a plethysmometer (MK-101P; Muromachi Kikai). When given on day 1, the injection usually induced the onset of chronic inflammation on day 9. The therapeutic regime started on day 10, after the signs of arthritis were obvious. All test samples, including naproxen, Lyprinol, eicosapentaenoic acid (EPA), and F-acids, were diluted with olive oil and administered orally for 5 d. Only olive oil was administered to the control group. The inhibitory activity against arthritic inflammation was measured based on the inhibition of swelling of the untreated left hind paw during days 10–15 after the administration of the adjuvant. The arthritis score was assessed based on paw/tail/nose/ear inflammation and overall conditions on a scale of 0–12 (Fig. S3). Each dataset was n = 18 for both the control and naproxen, n = 7 for Lyprinol, n = 8 for EPA and F_6 (1 mg/kg) and F_6 (10 mg/kg) ethyl esters, and n = 17 for F-acid ethyl ester (5 mg/kg) (7). All experiments were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals of the University of Shizuoka. Results are expressed as means ± SEM. Group comparisons of the paw volumes and arthritis scores were performed using the Student's t test and the Wilcoxon rank-sum test, respectively.

1. Glass RL, Krick TP, Echardt AE (1974) New series of fatty acids in northern pike (*Esox lucius*). *Lipids* 9:1004–1008.
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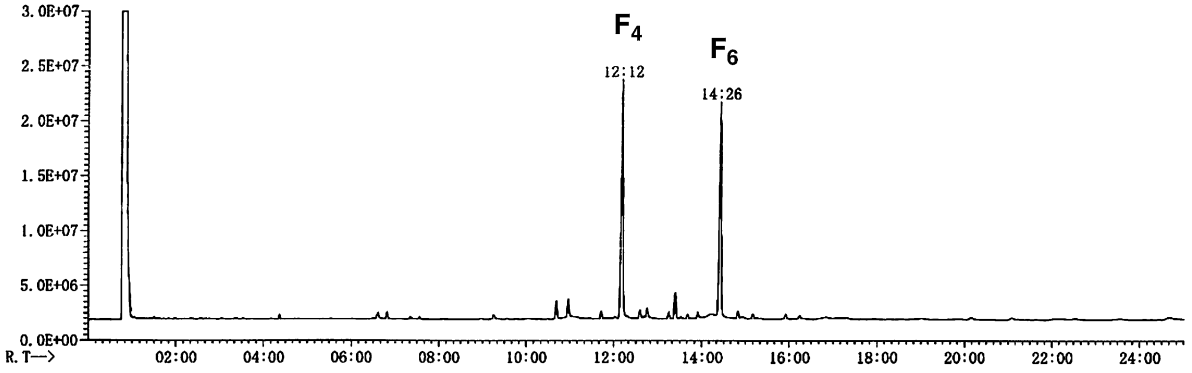

Fig. S1. GC-MS chart of F-acid fraction from Lyprinol.

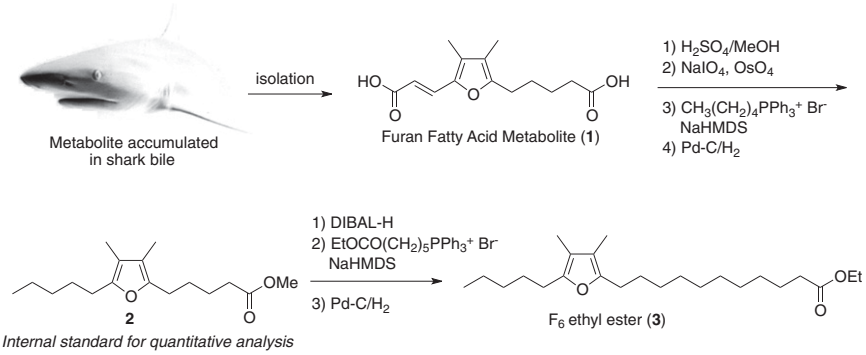

Fig. S2. Semisynthesis of furan fatty acid F_6.

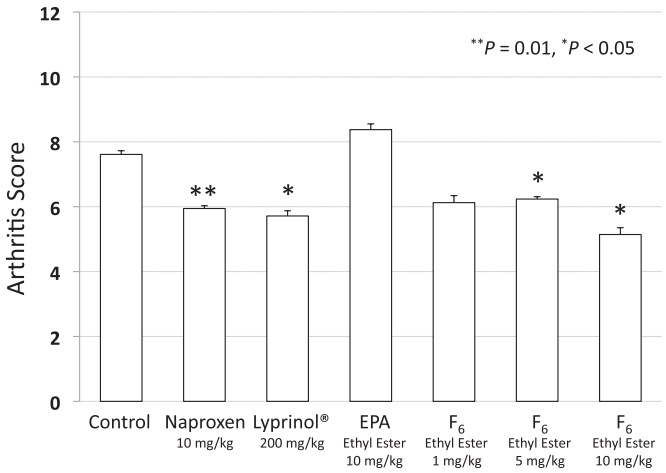

Fig. S3. Arthritis scores of the anti-inflammatory test. Error bars are SEM.

Table S1. Quantities of furan fatty acid methyl esters in Lyprinol

	F_4 methyl ester (mg/g)	F_6 methyl ester (mg/g)	Recovery rate (%)
No. 1	1.89	2.30	97.8
No. 2	2.00	2.23	97.5
No. 3	1.74	1.97	92.1
Mean ± SD	1.88 ± 0.13	2.17 ± 0.17	95.8 ± 3.2