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Anti-inflammatory Constituents of the Red Alga *Gracilaria verrucosa* and Their Synthetic Analogues

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A chemical study on the anti-inflammatory components of the red alga *Gracilaria verrucosa* led to the isolation of new 11-deoxyprostaglandins (**1**–**4**), a ceramide (**5**), and a C_{16} keto fatty acid (**6**), along with known oxygenated fatty acids (**7**–**14**). Their structures were elucidated on the basis of NMR and MS data. The absolute configurations of compounds **1**–**5** were determined by Mosher's method. The anti-inflammatory activity of the isolated compounds (**1**–**14**) was evaluated by determining their inhibitory effects on the production of pro-inflammatory mediators (NO, IL-6, and TNF-α) in lipopolysaccharide (LPS)-activated RAW 264.7 murine macrophage cells. Compounds **9** and **10** exhibited the most potent activity. In the evaluation of these two compounds and derivatized analogues (**15**−**40**), the anti-inflammatory activity was enhanced in some synthetic analogues. These enone fatty acids were investigated as potential anti-inflammatory leads for the first time.

Regulation of the inflammatory response is an essential element in the pathogenesis of chronic inflammation-related disorders. The inflammatory response involves the sequential activation of various signaling pathways, including cyclooxygenases, nitric oxide synthase, cytokines, and many more.[1] It is well-established that the excessive production of several pro-inflammatory mediators, including nitric oxide (NO), interleukins (IL-6 and IL-1β), and tumor necrosis factor-α (TNF-α), is implicated in several inflammatory-related diseases such as rheumatoid arthritis, inflammatory bowel disease, osteoarthritis, psoriasis, endotoxemia, and toxic shock syndrome.[2−12] Therefore, inhibition of the overproduction of these pro-inflammatory mediators should be a useful approach to treat these conditions.

The red alga *Gracilaria verrucosa* has been the subject of previous investigations due to the presence of prostaglandins such as PGA_2 and PGE_2.[13,14] It was also suggested that the high content of these prostaglandins in this alga was responsible for an associated food poisoning, known as Ogonori poisoning, in Japan.[15]

In our preliminary investigation of the alga for its biological activities, the methanolic extract was found to have significant inhibitory activity to the pro-inflammatory cytokines IL-6 and TNF-α. Therefore, the extract was subjected to bioassay-guided separation and resulted in the isolation of four 11-deoxyprostaglandins (**1**–**4**), a ceramide (**5**), and oxygenated fatty acids (**6**–**14**). 11-Deoxyprostaglandins were previously reported as synthetic analogues of natural prostaglandins and have various biological activities in their own right, such as bronchodilating, diuretic, and antigastric secretion activities.[16,17] Therefore, they have been the target for total synthesis dating back to the early days of prostaglandin research.[18−23] The anti-inflammatory activity of the isolated compounds (**1**−**14**) was evaluated by determining their inhibitory effects on the production of major pathophysiological mediators (NO, IL-6, and TNF-α) in lipopolysaccharide (LPS)-activated RAW 264.7 murine macrophage cells. Compounds **9** and **10** exhibited the most significant suppressive effect on the pro-inflammatory mediators. To further investigate the anti-inflammatory activity and the structure−activity relationship (SAR) of this type of fatty acid, compounds **9**, **10**, and their analogues (**15**–**40**) were synthesized. In the present study, we report the isolation and structural elucidation of the compounds **1**–**14** and the synthesis and biological evaluation of the enone fatty acids **9** and **10** and their synthetic analogues (**15**–**40**).

Results and Discussion

Compound **1** was isolated as a colorless oil. Its molecular formula was defined as $C_{20}H_{32}O_4$ on the basis of HRFABMS and NMR data. The exact mass of the $[M + Na]^+$ ion (*m/z* 359.2187) matched well with the expected molecular formula $C_{20}H_{32}O_4Na$ (Δ −1.1 mmu). The ^{13}C NMR spectrum exhibited 20 carbons that were attributed to one methyl (δ 14.4), 10 sp^3 methylenes (δ 38.7, 38.5, 35.2, 33.0, 28.9, 27.9, 26.4, 26.3, 25.8, and 23.8), three sp^3 methines (δ 73.6, 55.9, and 46.1), four olefinic carbons (δ 135.6, 134.4, 131.7, and 128.0), and two carbonyl carbons (δ 221.7 and 178.5). The downfield-shifted carbonyl carbon at δ 221.7 indicated the presence of the characteristic ketone function of the cyclopentanone ring.[24] The ^1H NMR spectrum of **1** disclosed two disubstituted olefins (δ 5.61, 5.53, 5.40, and 5.33), an allylic oxymethine (δ 4.00), and a terminal methyl group (δ 0.90). Analysis of the $^1H-^1H$ COSY spectrum demonstrated sequential correlations from H-2 (δ 2.26) to H-8 (δ 2.00) on the α-side chain, between H-8 and H-12, and from H-12 (δ 2.51) to H-20 (δ 0.90) on the ω-side chain. In the HMBC spectrum, key correlations from C-8 to H-7 and H-11, from C-9 to H-7, H-10, and H-11, and from C-12 to H-7, H-10, and H-14 were observed. Therefore, the planar structure of **1** was defined as 11-deoxyprostaglandin E_2. The double bond at C-5 was assigned the *Z* configuration (δ_C 27.9 and 25.8 for C-4 and C-7, respectively).[25] The double bond at C-13 was assigned the *E* configuration from the coupling constant between the corresponding protons ($J_{13,14}$ = 15.2 Hz). The *trans* stereochemistry of the α- and ω-side chains on the cyclopentanone ring was established by a NOE experiment.[26,27] The NOE correlation between H-7 and H-12 was observed in the NOESY spectrum. In the same manner, the orientation of the two side chains on the cyclopentanone ring of compounds **2**–**4** was also determined to be *trans*.

The absolute configuration at C-15 of **1** was defined by the Mosher's ester method. Compound **1** was treated overnight with (*S*)-(+)- and (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride in $CDCl_3/C_5D_5N$ at room temperature to afford the (*R*)-

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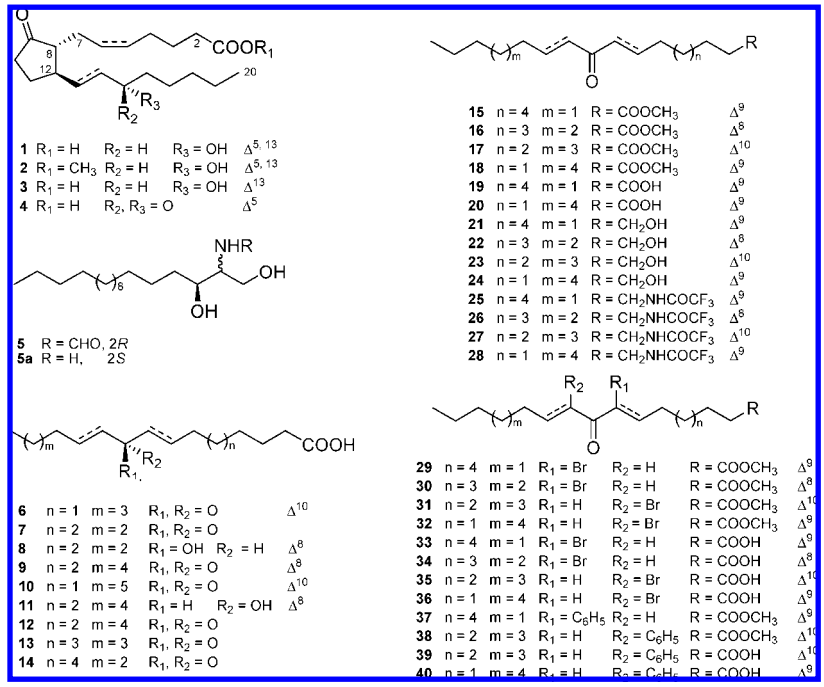
Chart 1.



and (*S*)-MTPA esters, respectively (**1r** and **1s**, respectively). Positive Δ_{SR} ($\Delta_S - \Delta_R$) values were observed for H-13 (+0.01) and H-14 (+0.01), while a negative value was observed for H-20 (−0.01), indicating that the configuration of the hydroxy group of **1** is *S* (Figure 1).

The *trans* orientation of the side chains in combination with the absolute configuration at C-15 (*S*) suggested that the absolute configuration of three chiral centers in **1** to be either (8*R*,12*R*,15*S*) or (8*S*,12*S*,15*S*). The synthetic (8*R*,12*R*,15*S*)-11-deoxyprostaglandin E_2 was reported to have a negative optical rotation ($[\alpha]_D$ −54.0),[18] while the (8*S*,12*S*,15*S*)-11-deoxyprostaglandin E_2 methyl ester was reported with a positive optical rotation ($[\alpha]_D$ +43.6).[19] Compound **1** showed a negative optical rotation ($[\alpha]_D$−33.0), and therefore, the absolute configuration of **1** was assigned as (8*R*,12*R*,15*S*), which is identical with that of common natural prostaglandins.

Compound **2** was isolated as a colorless oil. The molecular formula of $C_{21}H_{34}O_4$ was assigned by HRFABMS and NMR spectroscopic data. The exact mass of the $[M + Na]^+$ ion (*m*/*z* 373.2347) matched well with the expected molecular formula $C_{21}H_{34}O_4Na$ (Δ −0.8 mmu). The ^1H and ^{13}C NMR data of compound **2** were similar to those of **1** except for an additional methoxy group, showing the proton signal at δ 3.67 (3H, s) and the corresponding carbon signal at δ 51.2. The correlation between the methoxy group at δ_H 3.67 and the carbonyl carbon at δ_C 176.0 was observed in the HMBC spectrum, indicating that compound **2** is a methyl ester of **1**. The 15*S* configuration in **2** was determined on the basis of the similar ^{13}C NMR shifts of C-13−C-17 and optical rotation ($[\alpha]_D$ −35.0) in comparison with those of **1**. The synthetic (8*R*,12*R*,15*S*)-11-deoxyprostaglandin E_2 methyl ester and its (8*S*,12*S*,15*S*)-isomer were reported to show a negative ($[\alpha]_D$ −41) and a positive optical rotation ($[\alpha]_D$ +43.6), respectively.[19] Thus, the (8*R*,12*R*,15*S*) configuration was also established for **2**.

Compound **3** was isolated as a colorless oil. The HRFABMS and NMR data established the molecular formula of $C_{20}H_{34}O_4$ for **3**. The exact mass of the $[M + Na]^+$ ion (*m*/*z* 361.2358) matched well with the expected molecular formula $C_{20}H_{34}O_4Na$ (Δ +0.2 mmu). The ^1H NMR data of **3** were similar to those of **1**, except for the absence of the double bond in the α-side chain, leading to the upfield shift of the H-8 signal (δ 1.88) compared to those of **1** and **2** (δ 2.00). The data allowed assignment of the planar structure of **3** as 11-deoxyprostaglandin E_1. The 15*S* configuration was also determined on the basis of the similar ^{13}C NMR shifts of the carbons C-13 (δ 135.5), C-14 (δ 134.8), C-15 (δ 73.4), and C-16 (δ 38.7) in comparison with those of **1**. The synthetic (8*R*,12*R*,15*S*)-11-deoxyprostaglandin E_1 was reported to have a negative optical rotation ($[\alpha]_D$ −45.7).[21] Compound **3** showed the same sign of optical rotation ($[\alpha]_D$ −23.0). Therefore, as demonstrated for **1** and **2**, the absolute configuration of **3** was also defined as (8*R*,12*R*,15*S*).

Compound **4** was obtained as a colorless oil. The molecular formula of **4** was found to be $C_{20}H_{32}O_4$ by HRFABMS and NMR data. The exact mass of the $[M + Na]^+$ ion (*m*/*z* 359.2183) matched well with the expected molecular formula $C_{20}H_{32}O_4Na$ (Δ −1.5 mmu). The carbon signal at δ 213.6 indicated the presence of an additional keto group in **4**. In the COSY spectrum, a sequence of correlations from H-2 to H-8 on the α-side chain and another one starting from H-20 (δ 0.90) to H-16 (δ 2.46) were observed, indicating that the carbonyl carbon should be located at C-15. The *trans* orientation of the side chains on the cyclopentanone ring in conjunction with the negative optical rotation ($[\alpha]_D$ −53.0) confirmed the (8*R*,12*S*) configuration for **4**, identical to those of the reported compound ($[\alpha]_D$ −36.6), previously obtained by microbial transformation of PGA_2.[17h]

Compound **5** was isolated as a white, amorphous solid. The HRFABMS and NMR data revealed a molecular formula of $C_{19}H_{39}NO_3$. The exact mass of the $[M + Na]^+$ ion (*m*/*z* 352.2834) matched well with the expected molecular formula $C_{19}H_{39}NO_3Na$ (Δ +0.6 mmu). The ^1H NMR spectrum of **5** displayed a formamide group (δ 8.11),[28,29] an iminomethine (δ 3.90), a hydroxymethylene (δ 3.70), a hydroxymethine (δ 3.62), an intense aliphatic methylene band (δ 1.29), and a terminal methyl group (δ 0.90). The ^{13}C and DEPT spectra of **5** were supportive of the above analysis, showing a carbonyl (δ 163.8), an iminomethine (δ 55.6), a hydroxylmethylene (δ 62.0), a hydroxylmethine (δ 72.2), aliphatic methylenes (δ 23.7–34.8), and a terminal methyl carbon (δ 14.4). Analysis of 1D and 2D spectra disclosed a ceramide skeleton of **5**.

The absolute configuration of the hydroxymethine in **5** was defined by the Mosher's ester method. A negative Δ_{SR} ($\Delta_S - \Delta_R$) value was obtained for H-4 (−0.031), while a positive value was obtained for H-2 (+0.026), indicating that the configuration at C-3 is *S* (Figure 1). The remaining chiral center at C-2 was defined as *R* by comparison of the optical rotation of **5** with that of the synthetic L-*threo*-sphinganine (**5a**).[30] The synthetic model com-

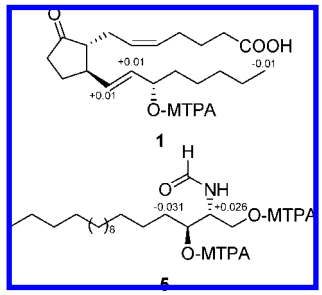


Figure 1. Δ_{SR} ($\Delta_S - \Delta_R$) values for the MTPA esters of compounds **1** and **5**.

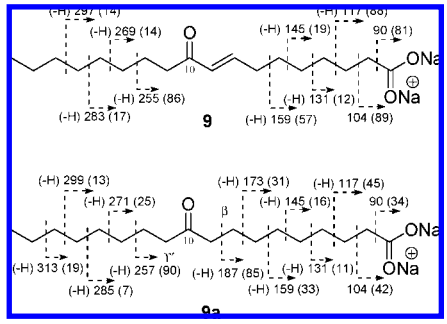


Figure 2. FAB-CID-MS/MS of $[M + 2Na - H]^+$ of **9** and its hydrogenated product **9a** with relative intensity values in parentheses.

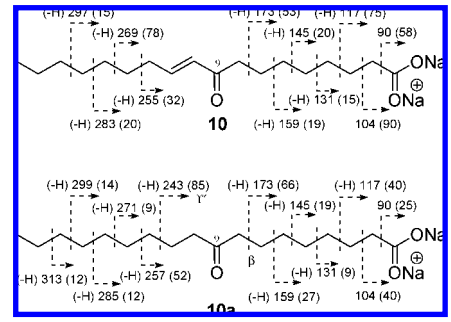


Figure 3. FAB-CID-MS/MS of $[M + 2Na - H]^+$ of **10** and its hydrogenated product **10a** with relative intensity values in parentheses.

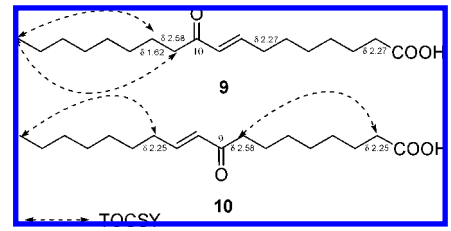


Figure 4. TOCSY correlations of compounds **9** and **10**.

pound **5a** with (2*S*,3*S*) configuration was reported to have a positive optical rotation ($[\alpha]_D$ +2.6), whereas compound **5** showed a negative optical rotation ($[\alpha]_D$ −2.7). Therefore, compound **5** was defined as (2*R*,3*S*)-2-formamido-1,3-dihydroxyoctadecane.

Compound **6** was obtained as a colorless oil. The molecular formula of $C_{16}H_{28}O_3$ was assigned for **6** on the basis of HRFABMS and ^{1}H NMR data. The exact mass of the $[M + Na]^+$ ion (*m*/*z* 291.1943) matched well with the expected molecular formula $C_{16}H_{28}O_3Na$ (Δ +0.7 mmu). The ^{1}H NMR data of **6** were almost identical to those of **9** and **10**, which were defined as enone fatty acids by a detailed analysis of 1D and 2D NMR data. Compounds **9** and **10** were previously produced by synthesis or biotransformation.[31,32] These two compounds were also uniquely found in airway cells of cotton workers and showed inhibitory effects on growth of several cancer cell lines.[33]

To locate the keto group as well as the double bond, compound **6** and other oxygenated fatty acids were analyzed by tandem mass spectrometry (FAB-CID-MS/MS). The structural determination of saturated oxo fatty acids was previously reported by utilizing charge-remote fragmentations (CRF) of "fixed" molecular ions such as $[M - H + 2Na]^+$.[34] The cationized acids undergo CRF upon high-energy activation, giving a product-ion pattern that has a gap corresponding to the oxo position and bordered by two high-intensity peaks. One of the peaks corresponds to an ion that is formed by the cleavage of the C−C bond β to the oxo position and proximal to the charge (β ion), whereas the other is formed from the cleavage of the C−C bond γ to the oxo position and distal to the charge (γ' ion). The oxo postion is easily determined by identifying the gap and the β and γ' ions (Figure 2). Applying this method to enone fatty acids (**9** and **10**), the CID spectra of $[M - H + 2Na]^+$ ions of these compounds also showed fragment ions along the chain and around the enone position. However, the definite location and direction of Figures 2 and 3 (either **9** or **10**) of the enone group could not be derived on the basis of these fragmentations. Therefore, to establish conclusively the location and direction of the enone group of compounds **6**, **9**, and **10**, these compounds were hydrogenated under palladium-charcoal (10% Pd-C) catalysis to give the corresponding keto fatty acids **6a**, **9a**, and **10a**, respectively. From the CID spectra of $[M - H + 2Na]^+$ ions of these hydrogenated products, the β ion was observed at *m*/*z* 173 for **6a** and **10a** and *m*/*z* 187 for **9a**; whereas, the γ' ion was found at *m*/*z* 243 for **6a** and **10a** and *m*/*z* 257 for **9a**. These diagnostic ions corroborated the location and direction of the enone group of **6**, **9**, and **10**. The CID spectra of $[M - H + 2Na]^+$ ions of the enone fatty acids (**9** and **10**) and their hydrogenated products (**9a** and **10a**, respectively) are shown in Figures 2 and 3.

Location of the two different enone moieties of compounds **6**, **9**, and **10** was further corroborated by TOCSY experiments. The correlations from H-2 to H-8 and from H-12 to the terminal methyl group were observed in the TOCSY spectra of **6** and **10**, whereas the correlation from H-18 (δ 0.92) to H-11 (δ 2.58) was observed in the TOCSY spectrum of **9** (Figure 4). Thus, the position of the double bond was located at C-10 for **6** and **10** and C-8 for **9**. As a result, compounds **6**, **9**, and **10** were defined as (*E*)-9-oxohexadec-10-enoic acid, (*E*)-10-oxooctadec-8-enoic acid, and (*E*)-9-oxooctadec-10-enoic acid, respectively.

Compounds **7** and **12**–**14**, identified as saturated keto fatty acids, were previously prepared from biotransformation or isolated from a few higher plants.[35–39] From the CID spectra of $[M - H + 2Na]^+$ ions of these compounds, the β ion of **7**, **12**, **13**, and **14** was observed at *m*/*z* 187, 187, 201, and 215, respectively, whereas the γ' ion of **7**, **12**, **13**, and **14** was found at *m*/*z* 257, 257, 271, and 285, respectively. These diagnostic ions led to the identification of the position of the keto group at C-10 for **7** and **12**, C-11 for **13**, and C-12 for **14**.

Compounds **8** and **11** were defined as monounsaturated monohydroxy fatty acids on the basis of 1D and 2D NMR data. The CID spectra of $[M - H + 2Na]^+$ ions of both compounds **8** and **11** showed a gap of 70 amu between two prominent peaks (*m*/*z* 159 and 229). These two peaks in turn had to be caused by allylic cleavage between C-6 and C-7 and cleavage adjacent to the hydroxy group between C-10 and C-11, respectively. The TOCSY spectrum of **11**, showing correlations from H-2 (δ 2.26) and H-3 (δ 1.59) to H-7 (δ 2.03), was supportive of this analysis. Therefore, the position of the double bond and the hydroxy group was located at C-8 and C-10, respectively. Compound **11** had an *R* configuration at C-10 since it showed the same sign of optical rotation ($[\alpha]_D$ −3.8) as that of the reported compound ($[\alpha]_D$ −1.48).[40] The absolute

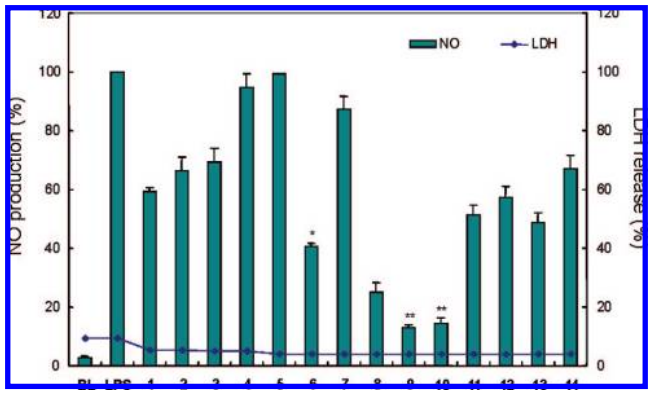


Figure 5. Inhibitory effects of the isolated compounds (**1**−**14**) on the production of NO in LPS-activated RAW 264.7 cells. RAW 264.7 cells (1.5×10^5 cells/mL) were stimulated with LPS (1 μg/mL) in the presence of test samples (20 μg/mL) for 24 h (BL, blank). Cell toxicity was determined using the LDH release method. Values are the mean ± SEM of three independent experiments performed in triplicate. *p < 0.05, **p < 0.01 compared with the LPS alone.

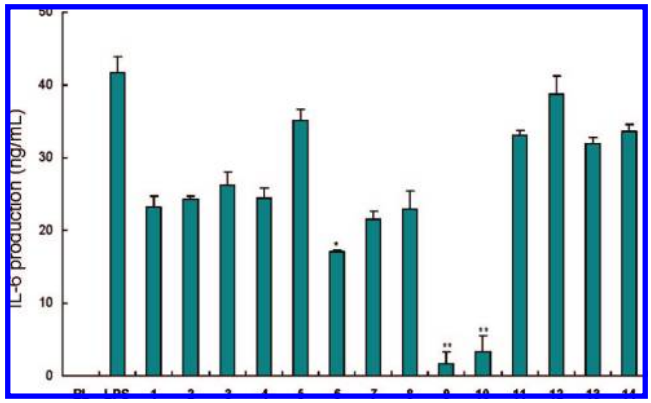


Figure 6. Inhibitory effects of the isolated compounds (**1**−**14**) on the production of IL-6 in LPS-activated RAW 264.7 cells. RAW 264.7 cells (8.0×10^5 cells/mL) were stimulated with LPS (1 μg/mL) in the presence of test samples (20 μg/mL) for 24 h (BL, blank). Values are the mean ± SEM of three independent experiments performed in triplicate. *p < 0.05, **p < 0.01 compared with the LPS alone.

configuration of the hydroxy group of **8**,[41] previously unassigned, was defined as *S* because it showed a positive optical rotation ($[\alpha]_D$ +2.8).

Compounds **1**–**14** were investigated for their anti-inflammatory activity through the evaluation of their inhibitory effects on the production of major pathophysiological mediators (NO, IL-6, and TNF-α) in murine macrophage cells (Figures 5, 6, and 7). The data showed that, among the compounds tested, compounds **9** and **10** produced the most significant effects on the pro-inflammatory mediators without notable cytotoxicity to the RAW 264.7 cells at their effective concentration. In a more detailed study, compounds **9** and **10** clearly showed suppressive effects on NO production (IC_{50} < 33.8 μM) and the expression of iNOS mRNA in a dose-dependent manner (data not shown). Therefore, to further investigate the anti-inflammatory activity and the structure−activity relationship (SAR) of this type of fatty acid, compounds **9** and **10** and their analogues (**15**−**40**) were synthesized. Structurally, compounds **9** and **10** possess a distinguished feature, an α,β-unsaturated carbonyl group (enone group), which has been encountered in a number of potential anti-inflammatory and anticancer agents such as sesquiterpene lactones (parthenolide),[42] 2-cyclopentenone-1-one and its derivatives,[43] and cyclopentenone prostaglandins.[44–48] Hence, the structural modification of **9** and **10** was directed to the enone group considering the electronic effect as well as the size and shape of substituents at the α-carbon. In parallel, the position of the enone group and the terminal functional group were modified.

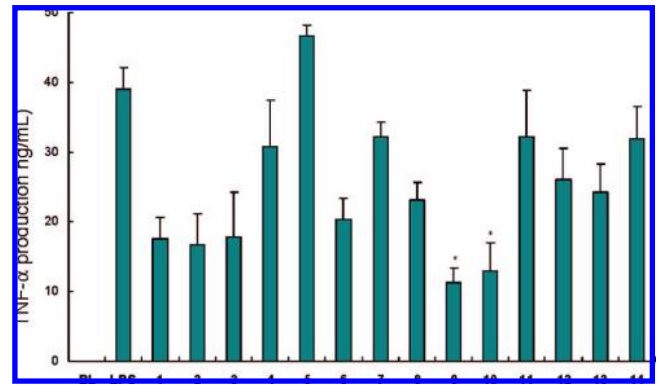
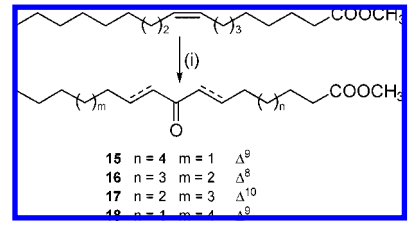


Figure 7. Inhibitory effects of the isolated compounds (**1**−**14**) on the production of TNF-α in LPS-activated RAW264.7 cells. RAW 264.7 cells (1.5×10^6 cells/mL) were stimulated with LPS (1 μg/mL) in the presence of test samples (20 μg/mL) for 6 h (BL, blank). Values are the mean ± SEM of three independent experiments performed in triplicate. *p < 0.05 compared with the LPS alone.

Scheme 1. Synthesis of Enone Fatty Acid Analogues (**15**–**18**)[a]

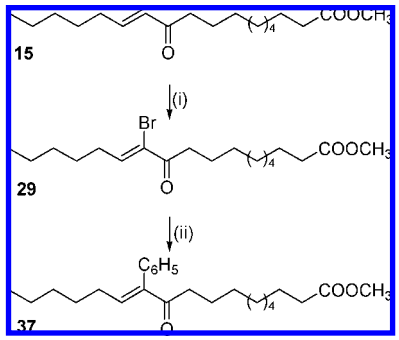


[a] Reagents and conditions: $Mn_3O(OAc)_9$, $(CH_3)_3COOH$, EtOAc, rt, 48 h.

The synthetic paths are outlined in Schemes 1 and 2. Scheme 1 describes the procedure of manganese(III) acetate-catalyzed allylic oxidation of methyl oleate to the corresponding enone fatty acid analogues (**15**–**18**). The compounds in other groups, including acid (**19**, **9**, **10**, and **20**), hydroxy (**21**–**24**), and amide groups (**25**−**28**), were prepared from different starting materials by the same process. Previously, the use of manganese(III) acetate as the catalyst for the allylic oxidation of cyclic alkenes to the corresponding enones was reported.[49] The simplicity and efficiency of this method in one reaction step led us to apply this protocol to prepare enones from acyclic alkenes such as oleic acid and its analogues. However, the direct oxidation of oleyl amine did not produce the corresponding enone products. This is probably because acidic condition are needed for this reaction.[49] Therefore, starting materials with functional groups such as ester, hydroxy, amide, and acid are suitable. From the oxidation of each starting material, four *trans* enone fatty acid isomers were formed with near equal yields, which were separated by a revered-phase HPLC (YMC ODS-H80) with the eluting order of **15** to **18** (Scheme 1). The structures of all synthetic enone fatty acids (**9**, **10**, and **15**–**28**) were secured mainly on the basis of their ^{1}H NMR and FAB-CID-MS/MS data.

To differentiate four enone fatty acid isomers in each group, all synthetic enone fatty acids were hydrogenated under palladium-charcoal (10% Pd-C) catalysis to furnish the corresponding keto fatty acid analogue, which was subjected to FAB-CID-MS/MS in order to identify the position of the keto group as described for the natural compounds. However, the hydrogenated products of four enone fatty acid isomers obtained from the oxidation of oleyl alcohol did not show clear fragmentations in their CID spectra.

Scheme 2. Synthesis of Compounds **29** and **37**[a]



[a] Reagents and conditions: (i) Br_2, CCl_4, rt, 3 h, then NH_4OH/ethanol, 3 h; (ii) $C_6H_7BO_2$, $Pd[(C_6H_5)_3P]_4$, 2M Na_2CO_3, ethanol, toluene, reflux, 3 h.

Therefore, these hydrogenated products were further oxidized to convert the primary hydroxy group to a carboxylic acid, which enabled location of the keto group of these compounds. Location of the two different enone moieties of the synthetic enone fatty acids was also corroborated by TOCSY experiments.

Scheme 2 outlines the synthetic path of the brominated and phenyl-substituted derivatives. The brominated analogues (**29**–**36**) were formed by the bromination of the enone fatty acids (**15**–**19**, **9**, **10**, and **20**) using Br_2 in CCl_4 followed by base-catalyzed elimination. Subsequent Suzuki coupling of some brominated compounds (**29**, **31**, **35**, and **36**) with phenylboronic acid mediated by tetrakis(triphenylphosphine)palladinium(0) afforded the corresponding phenyl-substituted analogues (**37**−**40**).

All of the synthetic compounds (**9**, **10**, and **15**−**40**) were examined for their anti-inflammatory activity through the evaluation of their inhibitory effects on the production of NO (Figure 8). When the activity of six synthetic groups was compared, the potency was in the order brominated (**29**−**36**) > hydroxy (**21**−**24**) > amide (**25**–**28**) > acid (**19**, **9**, **10**, and **20**) > methyl ester (**15**−**18**) > phenyl analogues (**37**−**40**). However, the increased activity of the brominated analogues, which were prepared to enhance the electrophilicity of the β-carbon,[50] was due to their strong cytotoxicity to the cells. Meanwhile, the phenyl-substituted analogues (**37**−**40**) did not show activity. Therefore, the presence of a bromine or a phenyl group at the α-carbon of the α,β-unsaturated carbonyl group appeared to be detrimental for the anti-inflammatory activity.

Regarding the role of the terminal functional group, the results indicated that the presence of a free acid group was also necessary for activity. However, substitution of the acid group by other functional groups such as hydroxy, amide, or amine might be desirable to enhance the activity. This indicates that, like cyclopentenone prostaglandins,[51] the presence of moieties other than the enone group might also be important in directing the enone reactivity toward targets, rendering enone fatty acids with enhanced selectivity.

Concerning the enone position, it was observed that the activity of the compounds having the double bond of the enone moiety distal to the terminal functional groups is stronger than that of those having the enone group in opposite direction, e.g., **10** > **9**; **20** > **19**; **23**, **24** > **21**, **22**; **27**, **28** > **25**, **26**. Taken together, the preliminary investigation of the structure−activity relationship of these enone fatty acids revealed that the reactive enone moiety still plays a vital role for the anti-inflammatory activity of these compounds. However, other factors such as the length of chain, the terminal functional group, and the position and direction of the enone group also modulate the activity of this type of fatty acid. Illustration of the structure−activity relationship of these enone fatty acids is presented in Figure 9.

The in vitro anti-inflammatory activity of this type of compound has been investigated for the first time and suggests that enone fatty acids may serve as potential anti-inflammatory leads.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO P-1020 digital polarimeter. ^1H, ^{13}C, and 2D NMR experiments were recorded on Varian Inova 400 and Varian Inova 500 spectrometers. Chemical shifts were reported with reference to the respective residual solvent or deuterated solvent peaks (δ_H 3.30 and δ_C 49.0 for CD_3OD, or δ_H 7.24 for $CDCl_3$). FABMS data were obtained on a JEOL JMS SX-102A spectrometer. HRFABMS data were obtained on JEOL JMS SX-101A spectrometer. HPLC was performed with a YMC ODS-H80 column (250 × 10 mm, 4 μm, 80 Å) and a C18-5E Shodex packed column (250 × 10 mm, 5 μm, 100 Å) using a Shodex RI-71 detector. Biological assays were performed at Cheju National University.

Extraction and Isolation. The red alga *Gracilaria verrucosa* (order Gigarinales, family Gracilariaceae) was collected off the coast of Jeju Island, South Korea. The alga was extracted with MeOH at room temperature, and the extract was partitioned between H_2O and CH_2Cl_2. The latter portion was further partitioned between *n*-hexane and aqueous MeOH. The aqueous MeOH fraction was subjected to a step-gradient reversed-phase flash column chromatography (YMC Gel ODS-A, 60 Å, 220 mesh) with a solvent system of 50 to 100% MeOH/H_2O to afford 20 fractions. Fraction 5 (136 mg), one of the bioactive fractions (5, 7, and 11) showing significant inhibitory activity against the pro-inflammatory cytokines, was subjected to a reversed-phase HPLC (C18-5E Shodex packed) eluting with 60% aqueous CH_3CN to afford compounds **1** (3.0 mg), **3** (1.0 mg), and **4** (7.0 mg). Fraction 7 (74 mg) was subjected to a reversed-phase HPLC (C18-5E Shodex packed) eluting with 81% aqueous MeOH to yield compounds **2** (2.0 mg), **6** (2 mg), **7** (1.5 mg), and **8** (4.0 mg). Compound **5** (1.2 mg) was obtained by separation of fraction 11 (125 mg) on a reversed-phase HPLC (YMC ODS-H80) eluting with 88% aqueous CH_3CN. Fraction 9 (238 mg) was subjected to a reversed-phase HPLC (C18-5E Shodex packed) eluting with 81% aqueous MeOH to afford compounds **9** (3.0 mg), **10** (3.2 mg), **11** (5.0 mg), **12** (1.5 mg), **13** (1.6 mg), and **14** (1.8 mg).

(5*Z*,13*E*)-(8*R*,12*R*,15*S*)-15-Hydroxy-9-oxoprosta-5,13-dienoic acid (1): colorless oil; $[\alpha]^{24}_D$ −33.0 (*c* 0.09, MeOH); ^1H NMR (CD_3OD, 400 MHz) δ 5.61 (1H, dd, J = 15.2, 7.6 Hz, H-13), 5.53 (1H, dd, J = 15.2, 6.8 Hz, H-14), 5.40 (1H, m, H-5), 5.33 (1H, m, H-6), 4.00 (1H, q, J = 6.8 Hz, H-15), 2.51 (1H, m, H-12), 2.33 (3H, m, H-7, H-10b), 2.26 (2H, t, J = 6.8 Hz, H-2), 2.10 (4H, m, H-4, H-11), 2.00 (1H, m, H-8), 1.65 (3H, m, H-3, H-10a), 1.54 (2H, m, H-16), 1.48–1.28 (6H, m, H-17-H-19), 0.90 (3H, t, J = 7.2 Hz, H-20); ^{13}C NMR (CD_3OD, 100 MHz) δ 221.7 (s, C-9), 178.5 (s, C-1), 135.6 (d, C-13), 134.4 (d, C-14), 131.7 (d, C-5), 128.0 (d, C-6), 73.6 (d, C-15), 55.9 (d, C-8), 46.1 (d, C-12), 38.7 (t, C-10), 38.5 (t, C-16), 35.2 (t, C-2), 33.0 (t, C-18), 28.9 (t, C-11), 27.9 (t, C-4), 26.4 (t, C-17), 26.3 (t, C-3), 25.8 (t, C-7), 23.8 (t, C-19), 14.4 (q, C-20); HRFABMS *m*/*z* 359.2187 [M + Na]$^+$ (calcd for $C_{20}H_{32}O_4Na$, 359.2198).

Preparation of the (*R*)- and (*S*)-MTPA Esters of 1. Two portions (each 0.4 mg) of compound **1** were treated overnight with (*S*)-(+)- and (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (1 μL) in $CDCl_3$ (0.5 mL)/C_5D_5N (4 μL) at room temperature to afford the (*R*)- and (*S*)-MTPA esters, respectively (**1r** and **1s**, respectively).

(*R*)-MTPA ester (1r): ^1H NMR ($CDCl_3$, 500 MHz) δ 5.935 (1H, dd, J = 16.0, 6.5 Hz, H-13), 5.785 (1H, dd, J = 16.0, 6.5 Hz, H-14), 5.42 (2H, m, H-5, H-6), 5.149 (1H, q, J = 6.5 Hz, H-15), 2.52–1.26 (22H, m), 0.85 (3H, t, J = 7.0 Hz, H-20).

(*S*)-MTPA ester (1s): ^1H NMR ($CDCl_3$, 500 MHz) δ 5.945 (1H, dd, J = 15.0, 6.5 Hz, H-13), 5.795 (1H, dd, J = 15.0, 6.5 Hz, H-14), 5.44 (2H, m, H-5, H-6), 5.165 (1H, q, J = 6.5 Hz, H-15), 2.48–1.21 (22H, m), 0.84 (3H, t, J = 6.5 Hz, H-20).

Methyl-(5*Z*,13*E*)-(8*R*,12*R*,15*S*)-15-hydroxy-9-oxoprosta-5,13-dienoate (2): colorless oil; $[\alpha]^{25}_D$ –35.0 (*c* 0.05, MeOH); ^1H NMR (CD_3OD, 500 MHz) δ 5.61 (1H, dd, J = 15.5, 7.6 Hz, H-13), 5.51 (1H, dd, J = 15.5, 6.5 Hz, H-14), 5.38 (1H, m, H-5), 5.32 (1H, m, H-6), 4.00 (1H, q, J = 6.5 Hz, H-15), 3.67 (3H, s, OCH_3), 2.51 (1H, m, H-12), 2.32 (3H, m, H-7, H-10b), 2.30 (2H, t, J = 7.2 Hz, H-2), 2.10 (4H, m, H-4, H-11), 2.00 (1H, m, H-8), 1.65 (3H, m, H-3, H-10a), 1.53 (2H, m, H-16), 1.46–1.28 (6H, m, H-17-H-19), 0.90 (3H, t, J = 7.0 Hz, H-20); ^{13}C NMR (CD_3OD, 100 MHz) δ 221.0 (s, C-9), 176.0 (s, C-1), 135.6 (d, C-13), 134.4 (d, C-14), 131.4 (d, C-5), 128.2 (d, C-6), 73.6 (d, C-15), 55.9 (d, C-8), 51.2 (q, OCH_3), 46.1 (d, C-12), 38.7 (t, C-10), 38.5 (t, C-16), 34.2 (t, C-2), 33.0 (t, C-18), 28.9 (t, C-11), 27.7 (t, C-4), 26.4 (t, C-17), 25.9 (t, C-3), 25.8 (t, C-7), 23.7(t,

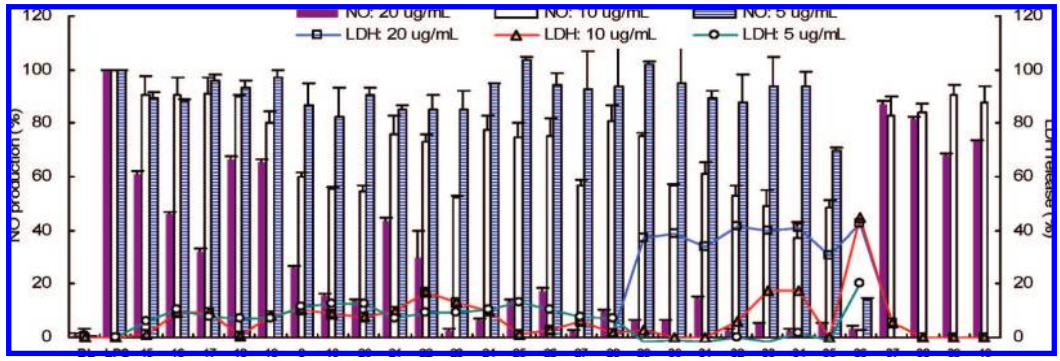


Figure 8. Inhibitory effects of the synthetic compounds (**9**, **10**, and **15**−**40**) on the production of NO in LPS-activated RAW 264.7 cells. RAW 264.7 cells (1.5×10^5 cells/mL) were stimulated with LPS (1 μg/mL) in the presence of test samples at concentrations of 5, 10, and 20 μg/mL for 24 h (BL, blank). Cell toxicity was determined using the LDH release method. Values are the mean ± SEM of three independent experiments performed in triplicate.

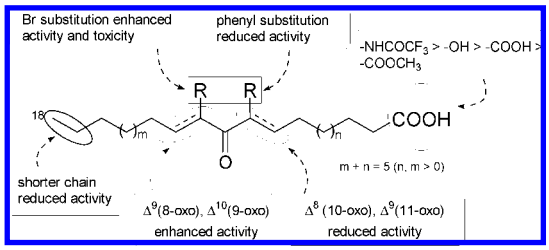


Figure 9. Structure–activity relationships of enone fatty acids. Anti-inflammatory activity was measured as inhibition on NO production.

C-19), 14.4 (q, C-20); HRFABMS *m/z*373.2347 [M + Na]$^+$ (calcd for $C_{21}H_{34}O_4Na$, 373.2355).

(*E*)-(8*R*,12*R*,15*S*)-15-Hydroxy-9-oxoprost-13-enoic acid (3): colorless oil; $[\alpha]^{27}_D$ −23.0 (*c* 0.04, MeOH); ^{1}H NMR (CD_3OD, 500 MHz) δ 5.61 (1H, dd, J = 15.5, 7.5 Hz, H-13), 5.51 (1H, dd, J = 15.5, 6.5 Hz, H-14), 4.00 (1H, q, J = 6.5 Hz, H-15), 2.48 (1H, m, H-12), 2.31 (1H, m, H-10b), 2.19 (2H, t, J = 7.5 Hz, H-2), 2.10 (2H, m, H-11), 1.88 (1H, m, H-8), 1.61 (3H, m, H-3, H-10a), 1.52 (2H, m, H-16), 1.43–1.28 (14H, m, CH_2), 0.90 (3H, t, J = 6.5 Hz, H-20); HRFABMS *m/z* 361.2358 [M + Na]$^+$ (calcd for $C_{20}H_{34}O_4Na$, 361.2356).

(*Z*)-(8*R*,12*S*)-9,15-Dioxoprost-5-enoic acid (4): colorless oil; $[\alpha]^{24}_D$ –53.0 (*c* 0.1, MeOH); ^{1}H NMR (CD_3OD, 500 MHz) δ 5.42 (1H, m, H-5), 5.33 (1H, m, H-6), 2.59 (1H, m, H-10b), 2.50 (1H, m, H-10a), 2.46 (2H, t, J = 7.2 Hz, H-16), 2.35 (2H, m, H-7), 2.28 (4H, t, J = 7.6 Hz, H-2, H-14), 2.11 (2H, q, J = 7.2 Hz, H-4), 2.02 (2H, m, H-11), 1.86 (2H, m, H-8, H-12), 1.64 (2H, quint, J = 7.2 Hz, H-3), 1.55 (6H, quint, J = 7.2 Hz, H-17-H-19), 1.45 (2H, m, H-13), 0.90 (3H, t, J = 7.2 Hz, H-20); ^{13}C NMR (CD_3OD, 100 MHz) δ 222.5 (s, C-9), 213.6 (s, C-15), 177.6 (s, C-1), 131.8 (d, C-5), 128.3 (d, C-6), 56.0 (d, C-8), 43.5 (t, C-16), 42.0 (d, C-12), 41.0 (t, C-10), 38.7 (t, C-14), 34.5 (t, C-2), 32.6 (t, C-18), 29.4 (t, C-13), 27.7 (t, C-11), 27.6 (t, C-4), 26.3 (t, C-7), 26.1 (t, C-3), 24.6 (t, C-17), 23.5 (t, C-19), 14.3 (q, C-20); HRFABMS *m/z* 359.2183 [M + Na]$^+$ (calcd for $C_{20}H_{32}O_4Na$, 359.2198).

(2*R*,3*S*)-2-Formamido-1,3-dihydroxyoctadecane (5): white, amorphous solid; $[\alpha]^{27}_D$ –2.7 (*c* 0.1, MeOH); ^{1}H NMR (CD_3OD, 400 MHz) δ 8.11 (1H, s, CHO), 3.90 (1H, q, J = 6.4 Hz, H-2), 3.70 (2H, d, J = 6.4 Hz, H-1), 3.62 (1H, m, H-3), 1.54 (2H, m, H-4), 1.29 (26H, brs, H-5-H-17), 0.90 (3H, t, J = 6.4 Hz, H-18); ^{13}C NMR (CD_3OD, 100 MHz) δ 163.8 (d, CHO), 72.2 (d, C-3), 62.0 (t, C-1), 55.6 (d, C-2), 34.8 (t, C-16), 33.1 (t, C-4), 30.8–30.5 (CH_2), 26.8 (t, C-15), 23.7 (t, C-17), 14.4 (q, C-18); HRFABMS *m/z* 352.2834 [M + Na]$^+$ (calcd for $C_{19}H_{39}NO_3Na$, 352.2828).

Preparation of the (*R*)- and (*S*)-MTPA Esters of 5. Two portions (each 0.2 mg) of compound **5** were treated overnight with (*S*)-(+)- and (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (0.4 μL) in $CDCl_3$ (0.5 mL)/C_5D_5N (2 μL) at room temperature to afford the (*R*)- and (*S*)-MTPA esters, respectively (**5r** and **5s**, respectively).

(*R*)-MTPA Ester (5r): ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.212 (1H, brs, NH), 4.562 (1H, dd, J = 11.6, 6.4 Hz, H-1b), 4.457 (1H, dd, J = 11.6, 3.2 Hz, H-1a), 4.217 (2H, m, H-2, H-3), 1.445 (2H, m, H-4), 1.225 (26H, brs, H-5-H-17), 0.849 (3H, t, J = 6.8 Hz, H-18).

(*S*)-MTPA ester (5s). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.303 (1H, brs, NH), 4.588 (1H, dd, J = 11.6, 6.4 Hz, H-1b), 4.430 (1H, dd, J = 11.6, 3.6 Hz, H-1a), 4.243 (2H, m, H-2, H-3), 1.414 (2H, m, H-4), 1.221 (26H, brs, H-5-H-17), 0.845 (3H, t, J = 6.4 Hz, H-18).

(*E*)-9-Oxohexadec-10-enoic acid (6): colorless oil; ^{1}H NMR (CD_3OD, 500 MHz) δ 6.94 (1H, dt, J = 14.0, 7.0 Hz, H-11), 6.13 (1H, dt, J = 14.0, 1.5 Hz, H-10), 2.60 (2H, t, J = 7.5 Hz, H-8), 2.26 (4H, m, H-2, H-12), 1.60 (4H, m, H-3, H-7), 1.51 (2H, quint, J = 7.0 Hz, H-13), 1.36–1.31 (10H, m, CH_2), 0.94 (3H, t, J = 7.0 Hz, H-16); FAB-CID-MS/MS *m/z* 313 [M – H + 2Na]$^+$ (100), 283 (5), 269 (29), 255 (20), 173 (52), 159 (31), 145 (12), 131 (7), 117 (33), 104 (33), 90 (29); HRFABMS *m/z* 291.1943 [M + Na]$^+$ (calcd for $C_{16}H_{28}NaO_3$, 291.1936).

9-Oxohexadecanoic acid (6a). To a stirred solution of **6** (0.6 mg) in MeOH was added 10% Pd/C (0.6 mg), and the resulting mixture was stirred at room temperature under an atmosphere of H_2 gas for 2 h. The mixture was filtered through Celite. The filtrate was concentrated under reduced pressure and purified by a reversed-phase HPLC (YMC ODS-H80) eluting with 90% aqueous MeOH to yield compound **6a** (0.3 mg): white, amorphous powder; FABMS *m/z*293 [M + Na]$^+$, *m/z* 315 [M – H + 2Na]$^+$; FAB-CID-MS/MS *m/z* 315 [M – H + 2Na]$^+$(100), 285 (10), 271 (9), 257 (50), 243 (86), 173 (66), 159 (27), 145 (20), 131 (9), 117 (45), 104 (40), 90 (21).

10-Oxohexadecanoic acid (7): white, amorphous powder; negative-ion FABMS *m/z*269 [M − H]$^-$; positive-ion FABMS *m/z* 293 [M + Na]$^+$, 315 [M – H + 2Na]$^+$; FAB-CID-MS/MS *m/z* 315 [M – H + 2Na]$^+$ (100), 299 (9), 285 (14), 271 (23), 257 (75), 187 (77), 173 (30), 159 (34), 145 (18), 131 (14), 117 (61), 104 (69), 90 (69).

(*E*)-(*S*)-10-Hydroxyhexadec-8-enoic acid (8): colorless oil; $[\alpha]^{24}_D$ +2.8 (*c* 0.04, MeOH); negative-ion FABMS *m/z* 269 [M − H]$^-$; positive-ion FABMS *m/z* 293 [M + Na]$^+$, 315 [M – H + 2Na]$^+$; FAB-CID-MS/MS *m/z* 315 [M – H + 2Na]$^+$ (100), 271 (12), 257 (10), 243 (14), 229 (20), 159 (24), 145 (12), 131 (10), 117 (78), 104 (90), 90 (71).

(*E*)-10-Oxooctadec-8-enoic acid (9): colorless oil; ^{1}H NMR (CD_3OD, 500 MHz) δ 6.94 (1H, dt, J = 16.0, 6.5 Hz, H-8), 6.13 (1H, dt, J = 16.0, 1.5 Hz, H-9), 2.58 (2H, t, J = 7.5 Hz, H-11), 2.27 (4H, m, H-2, H-7), 1.62 (4H, m, H-3, H-12), 1.51 (2H, m, H-6), 1.40−1.33 (14H, m, CH_2), 0.92 (3H, t, J = 6.5 Hz, H-18); FABMS *m/z* 319 [M + Na]$^+$, 341 [M – H + 2Na]$^+$; FAB-CID-MS/MS *m/z* 341 [M – H + 2Na]$^+$ (100), 297 (14), 283 (17), 269 (14), 255 (86), 159 (57), 145 (19), 131 (12), 117 (88), 104 (89), 90 (81).

10-Oxooctadecanoic acid (9a). The method of preparation of **9a** from **9** was similar to that used for the preparation of **6a**: white, amorphous powder; FABMS *m/z* 343 [M − H + 2Na]$^+$; FAB-CID-MS/MS *m/z* 343 [M – H + 2Na]$^+$(100), 313 (19), 299 (13), 285 (7), 271 (25), 257 (90), 187 (85), 173 (31), 159 (33), 145 (16), 131 (11), 117 (45), 104 (42), 90 (34).

(*E*)-9-Oxooctadec-10-enoic acid (10): colorless oil; ^{1}H NMR (CD_3OD, 500 MHz) δ 6.91 (1H, dt, J = 14.0, 7.0 Hz, H-11), 6.10 (1H, dt, J = 14.0, 1.5 Hz, H-10), 2.58 (2H, t, J = 7.5 Hz, H-8), 2.25 (4H, m, H-2, H-12), 1.58 (4H, m, H-3, H-7), 1.49 (2H, m, H-13),

1.33–1.28 (14H, m, CH_2), 0.90 (3H, t, J = 7.0 Hz, H-18); FABMS m/z 319 $[M + Na]^+$, 341 $[M - H + 2Na]^+$; FAB-CID-MS/MS m/z 341 $[M - H + 2Na]^+$ (100), 297 (15), 283 (20), 269 (78), 255 (32), 173 (53), 159 (19), 145 (20), 131 (15), 117 (75), 104 (90), 90 (58).

9-Oxooctadecanoic acid (10a). The method of preparation of **10a** from **10**was similar to that used for the preparation of **6a**: white, amorphous powder; FABMS m/z343 $[M - H + 2Na]^+$; FAB-CID-MS/MS m/z 343 $[M - H + 2Na]^+$ (100), 313 (12), 299 (14), 285 (12), 271 (9), 257 (52), 243 (85), 173 (66), 159 (27), 145 (19), 131 (9), 117 (40), 104 (40), 90 (25).

(*E*)-(*R*)-10-Hydroxyoctadec-8-enoic acid (11): colorless oil; $[\alpha]^{24}_D$ −3.8 (c 0.07, MeOH); negative-ion FABMS m/z 297 $[M - H]^-$; positive-ion FABMS m/z 321 $[M + Na]^+$, 343 $[M - H + 2Na]^+$; FAB-CID-MS/MS m/z 343 $[M - H + 2Na]^+$ (100), 299 (14), 271 (18), 257 (11), 243 (18), 229 (32), 159 (26), 145 (19), 131 (18), 117 (86), 104 (93), 90 (68).

10-Oxooctadecanoic acid (12): white, amorphous powder; negative-ion FABMS m/z 297 $[M - H]^-$; positive-ion FABMS m/z 321 $[M + Na]^+$, 343 $[M - H + 2Na]^+$; FAB-CID-MS/MS m/z 343 $[M - H + 2Na]^+$ (100), 327 (13), 313 (19), 299 (22), 285 (13), 271 (25), 257 (66), 187 (66), 173 (25), 159 (31), 145 (19), 131 (16), 117 (66), 104 (66), 90 (78).

11-Oxooctadecanoic acid (13): white, amorphous powder; negative-ion FABMS m/z 297 $[M - H]^-$; positive-ion FABMS m/z 321 $[M + Na]^+$, 343 $[M - H + 2Na]^+$; FAB-CID-MS/MS m/z 343 $[M - H + 2Na]^+$(100), 313 (14), 299 (14), 285 (36), 271 (71), 201 (75), 187 (25), 173 (32), 159 (29), 145 (21), 131 (14), 117 (71), 104 (89), 90 (64).

12-Oxooctadecanoic acid (14): white, amorphous powder; FABMS m/z 321 $[M + Na]^+$, 343 $[M - H + 2Na]^+$; FAB-CID-MS/MS m/z 343 $[M - H + 2Na]^+$ (100), 299 (16), 285 (42), 215 (42), 201 (29), 187 (24), 173 (21), 159 (26), 145 (21), 131 (13), 117 (63), 104 (63), 90 (55).

Preparation of 15–18. General Procedure. To a solution of methyl oleate (1.77 mmol), which was prepared from oleic acid, in EtOAc (5 mL) was added *tert*-butyl hydroperoxide in decane (8.85 mmol) and 3 Å molecular sieves (300 mg). The mixture was stirred for 30 min at room temperature. Manganese(III) acetate dihydrate (0.18 mmol) was added, and the mixture was stirred for 48 h at room temperature. The solution was diluted with ether and filtered through Celite. The filtrate was concentrated under reduced pressure and purified by a reversed-phase HPLC (YMC ODS-H80, 250 × 20 mm, 4 μm, 80 Å) eluting with 95% aqueous CH_3CN to yield compounds **15** (23 mg), **16** (25 mg), **17** (29 mg), and **18** (26 mg).

Methyl-(*E*)-11-oxooctadec-9-enoate (15): colorless oil; ^{1}H NMR (CD_3OD, 400 MHz) δ 6.91 (1H, dt, J = 16.0, 6.8 Hz, H-9), 6.11 (1H, dt, J = 16.0, 1.2 Hz, H-10), 3.64 (3H, s, OCH_3), 2.57 (2H, t, J = 7.6 Hz, H-12), 2.31 (2H, t, J = 7.6 Hz, H-2), 2.22 (2H, q, J = 6.8 Hz, H-8), 1.59 (4H, m, H-3, H-13), 1.49 (2H, m, H-7), 1.34–1.30 (14H, m, CH_2), 0.89 (3H, t, J = 6.8 Hz, H-18); FABMS m/z 333 $[M + Na]^+$.

Methyl-11-oxooctadecanoate (15a). The method of preparation of **15a** from **15** was similar to that used for the preparation of **6a**: white, amorphous powder; FABMS m/z335 $[M + Na]^+$; FAB-CID-MS/MS m/z 335 $[M + Na]^+$(100), 305 (9), 291 (9), 277 (16), 263 (80), 193 (51), 177 (25), 164 (14), 151 (5), 135 (5), 122 (4), 109 (10), 96 (13), 80 (9).

Methyl-(*E*)-10-oxooctadec-8-enoate (16): colorless oil; ^{1}H NMR (CD_3OD, 400 MHz) δ 6.91 (1H, dt, J = 16.4, 6.8 Hz, H-8), 6.11 (1H, dt, J = 16.4, 1.2 Hz, H-9), 3.64 (3H, s, OCH_3), 2.57 (2H, t, J = 7.6 Hz, H-11), 2.31 (2H, t, J = 7.2 Hz, H-2), 2.23 (2H, q, J = 6.8 Hz, H-7), 1.58 (4H, m, H-3, H-12), 1.49 (2H, m, H-6), 1.35–1.30 (14H, m, CH_2), 0.89 (3H, t, J = 6.8 Hz, H-18); FABMS m/z 333 $[M + Na]^+$.

Methyl-10-oxooctadecanoate (16a). The method of preparation of **16a** from **16** was similar to that used for the preparation of **6a**: white, amorphous powder; FABMS m/z335 $[M + Na]^+$; FAB-CID-MS/MS m/z 335 $[M + Na]^+$(100), 305 (8), 291 (9), 277 (5), 263 (23), 249 (89), 179 (52), 166 (10), 151 (7), 135 (5), 122 (3), 109 (7), 96 (8), 80 (7).

Methyl-(*E*)-9-oxooctadec-10-enoate (17): colorless oil; ^{1}H NMR (CD_3OD, 400 MHz) δ 6.92 (1H, dt, J = 16.0, 6.8 Hz, H-11), 6.10 (1H, dt, J = 16.0, 1.6 Hz, H-10), 3.64 (3H, s, OCH_3), 2.57 (2H, t, J = 7.2 Hz, H-8), 2.31 (2H, t, J = 7.2 Hz, H-2), 2.23 (2H, q, J = 6.8 Hz, H-12), 1.58 (4H, m, H-3, H-7), 1.48 (2H, m, H-13), 1.32–1.29 (14H, m, CH_2), 0.90 (3H, t, J = 7.6 Hz, H-18); FABMS m/z 333 $[M + Na]^+$.

Methyl-9-oxooctadecanoate (17a). The method of preparation of **17a** from **17** was similar to that used for the preparation of **6a**: white, amorphous powder; FABMS m/z 335 $[M + Na]^+$; FAB-CID-MS/MS m/z 335 $[M + Na]^+$(100), 305 (5), 291 (6), 277 (6), 263 (14), 249 (16), 235 (63), 165 (24), 151 (6), 135 (3), 122 (2), 109 (3), 96 (7), 80 (6).

Methyl-(*E*)-8-oxooctadec-9-enoate (18): colorless oil; ^{1}H NMR (CD_3OD, 400 MHz) δ 6.92 (1H, dt, J = 15.6, 6.8 Hz, H-10), 6.10 (1H, dt, J = 15.6, 1.6 Hz, H-9), 3.64 (3H, s, OCH_3), 2.58 (2H, t, J = 7.2 Hz, H-7), 2.31 (2H, t, J = 7.6 Hz, H-2), 2.23 (2H, q, J = 6.8 Hz, H-11), 1.58 (4H, m, H-3, H-6), 1.48 (2H, m, H-12), 1.33–1.28 (14H, m, CH_2), 0.89 (3H, t, J = 6.8 Hz, H-18); FABMS m/z 333 $[M + Na]^+$.

Methyl-8-oxooctadecanoate (18a). The method of preparation of **18a** from **18** was similar to that used for the preparation of **6a**: white, amorphous powder; FABMS m/z 335 $[M + Na]^+$; FAB-CID-MS/MS m/z 335 $[M + Na]^+$(100), 305 (8), 291 (10), 277 (8), 263 (19), 249 (13), 235 (19), 221 (91), 151 (24), 135 (14), 122 (3), 109 (7), 96 (5), 80 (5).

(*E*)-11-Oxooctadec-9-enoic acid (19). The method of preparation of the acid derivatives (**19**, **9**, **10**, and **20**) from oleic acid was similar to that used for the preaparion of the methyl ester analogues (**15**−**18**): colorless oil; ^{1}H NMR (CD_3OD, 400 MHz) δ 6.92 (1H, dt, J = 16.4, 7.2 Hz, H-9), 6.11 (1H, dt, J = 16.4, 1.2 Hz, H-10), 2.57 (2H, t, J = 7.6 Hz, H-12), 2.25 (4H, m, H-2, H-8), 1.59 (4H, m, H-3, H-13), 1.49 (2H, m, H-7), 1.34–1.30 (14H, m, CH_2), 0.89 (3H, t, J = 6.8 Hz, H-18); FABMS m/z 341 $[M + 2Na - H]^+$.

11-Oxooctadecanoic acid (19a). The method of preparation of **19a** from **19** was similar to that used for the preparation of **6a**: white, amorphous powder; FABMS m/z 343 $[M - H + 2Na]^+$; FAB-CID-MS/MS m/z 343 $[M - H + 2Na]^+$ (100), 313 (16), 299 (11), 285 (21), 271 (76), 201 (94), 187 (31), 173 (64), 159 (35), 145 (23), 131 (12), 117 (56), 104 (52), 90 (35).

(*E*)-8-Oxooctadec-9-enoic acid (20): colorless oil; ^{1}H NMR (CD_3OD, 400 MHz) δ 6.91 (1H, dt, J = 16.0, 7.6 Hz, H-10), 6.10 (1H, dt, J = 16.0, 1.4 Hz, H-9), 2.58 (2H, t, J = 7.2 Hz, H-7), 1.58 (4H, m, H-3, H-6), 1.46 (2H, m, H-12), 1.35–1.21 (14H, m, CH_2), 0.89 (3H, t, J = 6.8 Hz, H-18); FABMS m/z 341 $[M + 2Na - H]^+$.

8-Oxooctadecanoic acid (20a). The method of preparation of **20a** from **20**was similar to that used for the preparation of **6a**: white, amorphous powder; FABMS m/z 343 $[M - H + 2Na]^+$; FAB-CID-MS/MS m/z 343 $[M - H + 2Na]^+$(100), 313 (11), 299 (11), 285 (12), 271 (37), 257 (7), 243 (16), 229 (92), 159 (52), 145 (17), 131 (11), 117 (48), 104 (42), 90 (33).

(*E*)-11-Oxooctadec-9-enol (21). The method of preparation of the hydroxy analogues (**21**−**24**) from oleyl alcohol was similar to that used for the preparation of the methyl ester analogues (**15**−**18**): colorless oil; ^{1}H NMR (CD_3OD, 400 MHz) δ 6.92 (1H, dt, J = 15.6, 6.4 Hz, H-9), 6.10 (1H, dt, J = 15.6, 1.2 Hz, H-10), 3.53 (2H, t, J = 6.8 Hz, H-1), 2.57 (2H, t, J = 7.2 Hz, H-12), 2.25 (2H, q, J = 6.4 Hz, H-8), 1.59 (2H, m, H-13), 1.50 (4H, m, H-2, H-7), 1.34–1.28 (16H, m, CH_2), 0.89 (3H, t, J = 6.8 Hz, H-18); FABMS m/z 281 $[M - H]^-$.

(*E*)-10-Oxooctadec-8-enol (22): colorless oil; ^{1}H NMR (CD_3OD, 400 MHz) δ 6.92 (1H, dt, J = 16.0, 6.8 Hz, H-8), 6.10 (1H, dt, J = 16.0, 1.6 Hz, H-9), 3.53 (2H, t, J = 6.4 Hz, H-1), 2.58 (2H, t, J = 7.2 Hz, H-11), 2.25 (2H, q, J = 6.8 Hz, H-7), 1.58 (2H, m, H-12), 1.50 (4H, m, H-2, H-6), 1.37–1.30 (16H, m, CH_2), 0.89 (3H, t, J = 6.8 Hz, H-18); FABMS m/z 281 $[M - H]^-$.

(*E*)-9-Oxooctadec-10-enol (23): colorless oil; ^{1}H NMR (CD_3OD, 400 MHz) δ 6.93 (1H, dt, J = 16.4, 6.8 Hz, H-11), 6.10 (1H, dt, J = 16.4, 1.6 Hz, H-10), 3.53 (2H, t, J = 6.8 Hz, H-1), 2.58 (2H, t, J = 7.2 Hz, H-8), 2.25 (2H, q, J = 6.8 Hz, H-12), 1.58 (2H, m, H-7), 1.50 (4H, m, H-2, H-13), 1.33–1.29 (16H, m, CH_2), 0.89 (3H, t, J = 7.2 Hz, H-18); FABMS m/z 281 $[M - H]^-$.

(*E*)-8-Oxooctadec-9-enol (24): colorless oil; ^{1}H NMR (CD_3OD, 400 MHz) δ 6.92 (1H, dt, J = 16.0, 6.8 Hz, H-10), 6.10 (1H, dt, J = 16.0, 1.6 Hz, H-9), 3.53 (2H, t, J = 6.4 Hz, H-1), 2.58 (2H, t, J = 7.2 Hz, H-7), 2.24 (2H, q, J = 6.8 Hz, H-11), 1.58 (2H, m, H-6), 1.50 (4H, m, H-2, H-12), 1.33–1.28 (16H, m, CH_2), 0.89 (3H, t, J = 7.2 Hz, H-18); FABMS m/z 281 $[M - H]^-$.

Oxidation of the Hydrogenated Products of 21–24. The hydrogenated product **21a** or **22a**−**24a** (each 1 mg), which was formed by the hydrogenation of **21** or **22**–**24** by the same method described for the preparation of **6a**, was dissolved in acetone, and the solution was cooled to 0 °C. A 2 μL amount of Jones' reagent (6.68 g of CrO_3 in 5.75 mL of concentrated H_2SO_4, then H_2O until volume of 25 mL) was added, and the reaction was stirred at 0 °C for 2 h. The mixture was filtered and washed with diethyl ether. The filtrate was concentrated

under reduced pressure and purified by a reversed-phase HPLC (YMC ODS-H80) eluting with 95% aqueous MeOH to furnish keto fatty acids **21b**−**24b**. The ^{1}H NMR and MS data of **21b**, **22b**, **23b**, and **24b** were identical with those of **19a**, **9a**, **10a**, and **20a**, respectively.

(*E*)-*N*-Trifluoroacetyl-11-oxooctadec-9-enyl amide (25). The method of preparation of the amide analogues (**25**−**28**) from (*Z*)-*N*-trifluoroacetyl-octadec-9-enyl amide, which was prepared from oleyl amine and trifluoroacetic anhydride, was similar to that used for the preparation of the methyl ester analogues (**15**−**18**): colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.79 (1H, dt, J = 15.6, 6.8 Hz, H-9), 6.42 (1H, brs, NH), 6.06 (1H, d, J = 15.6 Hz, H-10), 3.33 (2H, q, J = 6.4 Hz, H-1), 2.49 (2H, t, J = 7.2 Hz, H-12), 2.18 (2H, q, J = 6.8 Hz, H-8), 1.55 (4H, m, H-2, H-13), 1.43 (2H, m, H-7), 1.28–1.17 (16H, m, CH_2), 0.85 (3H, t, J = 6.0 Hz, H-18); FABMS m/z 400 [M + Na]$^+$.

***N*-Trifluoroacetyl-11-oxooctadecanyl amide (25a).** The method of preparation of **25a** from **25** was similar to that used for the preparation of **6a**: white, amorphous powder; FABMS m/z 402 [M + Na]$^+$; FAB-CID-MS/MS m/z 402 [M + Na]$^+$ (100), 372 (12), 358 (9), 344 (21), 330 (71), 260 (68), 247 (27), 233 (14), 218 (12), 204 (10), 190 (7), 177 (9), 163 (9), 149 (14), 135 (3).

(*E*)-*N*-Trifluoroacetyl-10-oxooctadec-8-enyl amide (26): colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.79 (1H, dt, J = 15.6, 7.2 Hz, H-8), 6.50 (1H, brs, NH), 6.05 (1H, dt, J = 15.6, 1.2 Hz, H-9), 3.33 (2H, q, J = 6.8 Hz, H-1), 2.49 (2H, t, J = 7.6 Hz, H-11), 2.18 (2H, q, J = 7.2 Hz, H-7), 1.55 (4H, m, H-2, H-12), 1.43 (2H, m, H-6), 1.29–1.19 (16H, m, CH_2), 0.85 (3H, t, J = 6.8 Hz, H-18); FABMS m/z400 [M + Na]$^+$.

***N*-Trifluoroacetyl-10-oxooctadecanyl amide (26a).** The method of preparation of **26a** from **26** was similar to that used for the preparation of **6a**: white, amorphous powder; FABMS m/z 402 [M + Na]$^+$; FAB-CID-MS/MS m/z 402 [M + Na]$^+$ (100), 372 (9), 358 (10), 344 (5), 330 (22), 316 (85), 246 (68), 233 (21), 218 (9), 204 (8), 190 (9), 177 (9), 163 (7), 149 (14), 135 (4).

(*E*)-*N*-Trifluoroacetyl-9-oxooctadec-10-enyl amide (27): colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.79 (1H, dt, J = 16.0, 6.8 Hz, H-11), 6.45 (1H, brs, NH), 6.06 (1H, d, J = 16.0 Hz, H-10), 3.33 (2H, q, J = 6.8 Hz, H-1), 2.49 (2H, t, J = 7.2 Hz, H-8), 2.18 (2H, q, J = 6.8 Hz, H-12), 1.56 (4H, m, H-2, H-7), 1.42 (2H, m, H-13), 1.28–1.18 (16H, m, CH_2), 0.85 (3H, t, J = 6.8 Hz, H-18); FABMS m/z400 [M + Na]$^+$.

***N*-Trifluoroacetyl-9-oxooctadecanyl amide (27a).** The method of preparation of **27a** from **27** was similar to that used for the preparation of **6a**: white, amorphous powder; FABMS m/z402 [M + Na]$^+$; FAB-CID-MS/MS m/z 402 [M + Na]$^+$ (100), 372 (10), 358 (12), 344 (9), 330 (8), 316 (52), 302 (88), 232 (61), 218 (19), 204 (9), 190 (9), 177 (5), 163 (8), 149 (10), 135 (3).

(*E*)-*N*-Trifluoroacetyl-8-oxooctadec-9-enyl amide (28): colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.80 (1H, dt, J = 16.4, 6.8 Hz, H-10), 6.44 (1H, brs, NH), 6.06 (1H, d, J = 16.4 Hz, H-9), 3.33 (2H, q, J = 6.4 Hz, H-1), 2.50 (2H, t, J = 7.2 Hz, H-7), 2.18 (2H, q, J = 6.8 Hz, H-11), 1.56 (4H, m, H-2, H-6), 1.43 (2H, m, H-12), 1.30–1.20 (16H, m, CH_2), 0.85 (3H, t, J = 6.8 Hz, H-18); FABMS m/z 400 [M + Na]$^+$.

***N*-Trifluoroacetyl-8-oxooctadecanyl amide (28a).** The method of preparation of **28a** from **28** was similar to that used for the preparation of **6a**: white, amorphous powder; FABMS m/z402 [M + Na]$^+$; FAB-CID-MS/MS m/z 402 [M + Na]$^+$ (100), 372 (8), 358 (10), 344 (11), 330 (28), 316 (11), 302 (17), 288 (90), 218 (47), 205 (15), 190 (5), 177 (4), 163 (5), 149 (11), 135 (4).

Methyl-(*Z*)-10-bromo-11-oxooctadec-9-enoate (29). To a solution of **15** (20 mg) in CCl_4 (0.5 mL) was added bromine (4 μL), and the reaction was stirred at room temperature for 3 h. The solvent was removed at reduced pressure, and the residue was dissolved in 0.5 mL of EtOH, to which was added 50 μL of 28% NH_4OH. The resulting mixture was stirred for a further 3 h. After removal of solvent, the residue was subjected to a reversed-phase HPLC (YMC ODS-H80) eluting with 95% aqueous CH_3CN to yield compound **29** (6 mg): colorless oil; ^{1}H NMR (CD_3OD, 400 MHz) δ 7.30 (1H, t, J = 6.8 Hz, H-9), 3.64 (3H, s, OCH_3), 2.81 (2H, t, J = 7.6 Hz, H-12), 2.42 (2H, q, J = 6.8 Hz, H-8), 2.31 (2H, t, J = 7.6 Hz, H-2), 1.60–1.53 (6H, m, H-3, H-7, H-13), 1.36–1.31 (14H, m, CH_2), 0.89 (3H, t, J = 6.8 Hz, H-18); FABMS m/z 411/413 [M + Na]$^+$.

Methyl-(*Z*)-9-bromo-10-oxooctadec-8-enoate (30). The method of preparation of **30** from **16** was similar to that used for the preparation of **29**: colorless oil; ^{1}H NMR (CD_3OD, 400 MHz) δ 7.30 (1H, t, J = 7.2 Hz, H-8), 3.64 (3H, s, OCH_3), 2.81 (2H, t, J = 7.2 Hz, H-11), 2.42 (2H, q, J = 7.2 Hz, H-7), 2.32 (2H, t, J = 7.6 Hz, H-2), 1.63–1.54 (6H, m, H-3, H-6, H-12), 1.38–1.31 (14H, m, CH_2), 0.89 (3H, t, J = 6.8 Hz, H-18); FABMS m/z 411/413 [M + Na]$^+$.

Methyl-(*Z*)-10-bromo-9-oxooctadec-10-enoate (31). The method of preparation of **31** from **17** was similar to that used for the preparation of **29**: colorless oil; ^{1}H NMR (CD_3OD, 400 MHz) δ 7.31 (1H, t, J = 6.8 Hz, H-11), 3.64 (3H, s, OCH_3), 2.82 (2H, t, J = 7.6 Hz, H-8), 2.43 (2H, q, J = 6.8 Hz, H-12), 2.31 (2H, t, J = 8.0 Hz, H-2), 1.60–1.51 (6H, m, H-3, H-7, H-13), 1.35–1.31 (14H, m, CH_2), 0.90 (3H, t, J = 7.2 Hz, H-18); FABMS m/z 411/413 [M + Na]$^+$.

Methyl-(*Z*)-9-bromo-8-oxooctadec-9-enoate (32). The method of preparation of **32** from **18** was similar to that used for the preparation of **29**: colorless oil; ^{1}H NMR (CD_3OD, 400 MHz) δ 7.31 (1H, t, J = 6.8 Hz, H-10), 3.64 (3H, s, OCH_3), 2.82 (2H, t, J = 7.6 Hz, H-7), 2.43 (2H, q, J = 6.8 Hz, H-11), 2.31 (2H, t, J = 7.2 Hz, H-2), 1.63–1.53 (6H, m, H-3, H-6, H-12), 1.35–1.28 (14H, m, CH_2), 0.89 (3H, t, J = 6.8 Hz, H-18); FABMS m/z 411/413 [M + Na]$^+$.

(*Z*)-10-Bromo-11-oxooctadec-9-enoic acid (33). The method of preparation of **33** from **19** was similar to that used for the preparation of **29**: colorless oil; ^{1}H NMR (CD_3OD, 400 MHz) δ 7.31 (1H, t, J = 7.2 Hz, H-9), 2.81 (2H, t, J = 7.6 Hz, H-12), 2.43 (2H, q, J = 7.2 Hz, H-8), 2.27 (2H, t, J = 7.6 Hz, H-2), 1.60–1.53 (6H, m, H-3, H-7, H-13), 1.37–1.31 (14H, m, CH_2), 0.90 (3H, t, J = 7.6 Hz, H-18); FABMS m/z 397/399 [M + Na]$^+$.

(*Z*)-9-Bromo-10-oxooctadec-8-enoic acid (34). The method of preparation of **34** from **9** was similar to that used for the preparation of **29**: colorless oil; ^{1}H NMR (CD_3OD, 400 MHz) δ 7.31 (1H, t, J = 7.2 Hz, H-8), 2.81 (2H, t, J = 7.2 Hz, H-11), 2.43 (2H, q, J = 7.2 Hz, H-7), 2.27 (2H, t, J = 7.6 Hz, H-2), 1.62–1.53 (6H, m, H-3, H-6, H-12), 1.37–1.31 (14H, m, CH_2), 0.90 (3H, t, J = 7.6 Hz, H-18); FABMS m/z 397/399 [M + Na]$^+$.

(*Z*)-10-Bromo-9-oxooctadec-10-enoic acid (35). The method of preparation of **35** from **10** was similar to that used for the preparation of **29**: colorless oil; ^{1}H NMR (CD_3OD, 400 MHz) δ 7.30 (1H, t, J = 6.8 Hz, H-11), 2.81 (2H, t, J = 7.2 Hz, H-8), 2.42 (2H, q, J = 6.8 Hz, H-12), 2.27 (2H, t, J = 7.2 Hz, H-2), 1.63–1.53 (6H, m, H-3, H-7, H-13), 1.34–1.31 (14H, m, CH_2), 0.90 (3H, t, J = 6.8 Hz, H-18); FABMS m/z 397/399 [M + Na]$^+$.

(*Z*)-9-Bromo-8-oxooctadec-9-enoic acid (36). The method of preparation of **36** from **20** was similar to that used for the preparation of **29**: colorless oil; ^{1}H NMR (CD_3OD, 400 MHz) δ 7.31 (1H, t, J = 7.2 Hz, H-10), 2.82 (2H, t, J = 7.2 Hz, H-7), 2.43 (2H, q, J = 7.2 Hz, H-11), 2.28 (2H, t, J = 7.2 Hz, H-2), 1.63–1.51 (6H, m, H-3, H-6, H-12), 1.36–1.31 (14H, m, CH_2), 0.90 (3H, t, J = 6.8 Hz, H-18); FABMS m/z 397/399 [M + Na]$^+$.

Methyl-(*Z*)-10-phenyl-11-oxooctadec-9-enoate (37). To a stirred solution of **29** (5 mg) and 1.6 mg of phenylboronic acid in a mixture solvent of 1 mL of toluene, 1 mL of EtOH, and 35 μL of 2 M Na_2CO_3 was added 1 mg of tetrakis(triphenylphosphine)palladium(0). After refluxing with vigorous stirring for 3 h, the solvent was removed in vacuo. The residue was purified by a reversed-phase HPLC (YMC ODS-H80) eluting with 95% aqueous CH_3CN to yield compound **37** (2 mg): colorless oil; ^{1}H NMR (CD_3OD, 400 MHz) δ 7.40–7.30 (4H, m, Ar-H), 7.06 (1H, dd, J = 6.8, 1.6 Hz, Ar-H), 6.94 (1H, t, J = 7.2 Hz, H-9), 3.64 (3H, s, OCH_3), 2.61 (2H, t, J = 7.2 Hz, H-12), 2.27 (2H, t, J = 7.6 Hz, H-2), 2.05 (2H, q, J = 7.2 Hz, H-8), 1.55 (4H, m, H-3, H-13), 1.43 (2H, m, H-7), 1.28–1.22 (14H, m, CH_2), 0.88 (3H, t, J = 7.6 Hz, H-18); FABMS m/z 409 [M + Na]$^+$.

Methyl-(*Z*)-10-phenyl-9-oxooctadec-10-enoate (38). The method of preparation of **38** from **31** was similar to that used for the preparation of **37**: colorless oil; ^{1}H NMR (CD_3OD, 400 MHz) δ 7.40–7.30 (4H, m, Ar-H), 7.07 (1H, dd, J = 6.8, 1.2 Hz, Ar-H), 6.95 (1H, t, J = 7.6 Hz, H-11), 3.64 (3H, s, OCH_3), 2.62 (2H, t, J = 7.2 Hz, H-8), 2.30 (2H, t, J = 7.2 Hz, H-2), 2.05 (2H, q, J = 7.6 Hz, H-12), 1.57 (4H, m, H-3, H-7), 1.43 (2H, m, H-13), 1.29–1.22 (14H, m, CH_2), 0.87 (3H, t, J = 6.8 Hz, H-18); FABMS m/z409 [M + Na]$^+$.

(*Z*)-10-Phenyl-9-oxooctadec-10-enoic acid (39). The method of preparation of **39** from **35** was similar to that used for the preparation of **37**: colorless oil; ^{1}H NMR (CD_3OD, 400 MHz) δ 7.40–7.30 (4H, m, Ar-H), 7.07 (1H, dd, J = 6.8, 1.6 Hz, Ar-H), 6.95 (1H, t, J = 7.6 Hz, H-11), 2.62 (2H, t, J = 7.6 Hz, H-8), 2.25 (2H, t, J = 7.2 Hz, H-2), 2.05 (2H, q, J = 7.6 Hz, H-12), 1.57 (4H, m, H-3, H-7), 1.43 (2H, m, H-13), 1.30–1.21 (14H, m, CH_2), 0.87 (3H, t, J = 6.8 Hz, H-18); FABMS m/z 395 [M + Na]$^+$.

(*Z*)-9-Phenyl-8-oxooctadec-9-enoic acid (40). The method of preparation of **40** from **36** was similar to that used for the preparation

of **37**: colorless oil; ^{1}H NMR (CD_3OD, 400 MHz) δ 7.40–7.30 (4H, m, Ar-H), 7.07 (1H, dd, J = 6.8, 2.0 Hz, Ar-H), 6.95 (1H, t, J = 7.6 Hz, H-10), 2.62 (2H, t, J = 7.6 Hz, H-7), 2.25 (2H, t, J = 7.2 Hz, H-2), 2.04 (2H, q, J = 7.6 Hz, H-11), 1.57 (4H, m, H-3, H-6), 1.43 (2H, m, H-12), 1.31–1.21 (14H, m, CH_2), 0.88 (3H, t, J = 6.8 Hz, H-18); FABMS m/z 395 $[M + Na]^+$.

Cell Culture. The mouse macrophage RAW 264.7 was purchased from ATCC (Rockville, MD) and cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) heat-activated fetal bovine serum, streptomycin (100 μg/mL), and penicillin (100 U/mL) at 37 °C atmosphere and 5% CO_2.

Cytotoxicity Assay. Lactate dehydrogenase leakage is a means of measuring membrane integrity as a function of the amount of cytoplasmic LDH released from the cytosol into the medium. LDH activity was determined following the production of NADH during the conversion of lactate to pyruvate.[52] The release of LDH from RAW 264.7 cells was used to detect cytotoxicity and was measured at the end of each proliferation experiment. Briefly, culture medium was centrifuged at 12 000 rpm for 3 min at room temperature to ensure accumulation of cells. The cell-free culture medium (50 μg/mL) was collected and then incubated with 50 μL of the reaction mixture cytotoxicity detection kit for 30 min at room temperature in the dark. Then 1 N HCl (50 μL) was added into each well to stop the enzymatic reaction. The optical density of the solution was then measured by using an ELISA plate reader at 490 nm. Percent cytotoxicity was determined relative to the control group.

Nitrite Assay. The production of nitric oxide (NO) was measured, as previously described by Ryu et al.,[53] by using the Griess reagent (Sigma, MO). Briefly, the RAW 264.7 cells were stimulated with LPS (1 μg/mL), and 100 μL of the supernatant was mixed with 100 μL of the Griess reagent (0.1% naphthlyene diamine dihydrochloride, 1% sulfanilamide, 2.5% H_3PO_4). This mixture was incubated for 10 min at room temperature (light protected). Absorbance at 540 nm was measured using an ELISA reader (Amersham Pharmacia Biotech, UK), and the results were compared against a calibration curve using sodium nitrite as the standard.

Measurement of the Production of Pro-inflammatory Cytokines (IL-6 and TNF-α). The inhibitory effects of the isolated and synthetic compounds on IL-6 and TNF-α production were determined by the method previously described.[54] The samples were dissolved with EtOH and diluted with DMEM. The final concentration of chemical solvents did not exceed 0.1% in the culture medium. At these conditions, none of the solubilized solvents altered IL-6 and TNF-α production in RAW 264.7 cells. Before stimulation with LPS (1 μg/mL) and test materials, RAW 264.7 cells were incubated for 18 h in 24-well plates under the same conditions. Lipopolysaccharide (LPS) and the test materials were then added to the cultured cells. The medium was used for IL-6 and TNF-α assay using mouse ELISA kits (R & D Systems Inc., MN).

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