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New jasmonate analogues as potential anti-inflammatory agents

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ABSTRACT

In an effort to develop new anti-inflammatory agents, methyl jasmonate analogues (**2**–**20**) were synthesized and evaluated for their inhibitory effects on the production of pro-inflammatory mediators (NO, IL-6, and TNF-α) in lipopolysaccharide (LPS)-activated RAW264.7 murine macrophage cells. The introduction of an enone functionality to the structure of a plant hormone (**1**) rendered the product (**2**) a significant anti-inflammatory activity. Analogues further derived from **2** (**7**, **9**, **13**, and **15**) exhibited even more enhanced activity, and these compounds were much more potent than natural anti-inflammatory prostaglandins (PGA_1, PGA_2, and 15-deoxy-$\Delta^{12,14}$-PGJ_2). Among them, compounds **9** and **15** showed the highest potency, while compounds **7** and **13** would be more desirable with respect to safety. This is the first study demonstrating the anti-inflammatory potential of jasmonate derivatives, and the present results suggest that α-haloenone jasmonates (**7**, **9**, **13**, and **15**) may serve as potential anti-inflammatory leads.

1. Introduction

In our previous study on the anti-inflammatory components of the red alga *Gracilaria verrucosa*, several new prostaglandins and oxygenated fatty acids were isolated. Among them, enone fatty acids were confirmed as causative components for the inhibitory effect on the production and gene expression of pro-inflammatory mediators (NO, IL-6, and TNF-α) in lipopolysaccharide (LPS)-activated RAW264.7 murine macrophage cells. Thereafter, analogues of these enone fatty acids were synthesized and evaluated for anti-inflammatory activity and cytotoxicity.[1] On the basis of this study, we found that the α,β-unsaturated ketone (enone) functionality is essential for the activity. This finding has made us to engage in a study on anti-inflammatory and/or anti-tumor compounds with enone functionality, as exemplified by sesquiterpene lactones (parthenolide),[2] cyclopentenone prostaglandins (PGA_1, PGA_2, and 15-deoxy-$\Delta^{12,14}$-PGJ_2),[3–12] and oleanane tritepenoids (CDDO).[13–16] In a quest of new leads with the enone functionality and higher potency than natural anti-inflammatory prostaglandins (PGA_1, PGA_2, and 15-deoxy-$\Delta^{12,14}$-PGJ_2), we intended to perform structural modification on prostaglandin analogues isolated from *G. verrucosa*. However, due to limited amounts of the natural samples, methyl jasmonate (**1**), a plant hormone, which shares the same partial structure with anti-inflammatory prostaglandins, was selected as a starting material for the synthesis of cyclopentenone prostaglandin-like compounds.

Jasmonic acid and methyl jasmonate (**1**) are fatty acid-derived cyclopentanones occurring throughout the plant kingdom. Biosynthesis of jasmonates in plants is distinct from that of prostaglandins in mammals. In animals, prostaglandins are synthesized from arachidonic acid (C20:4), while jasmonates are synthesized from linolenic acid (C18:3) in plants.[17–19] Jasmonates in plants play major role in a defense against insects and disease. Leaf tissues exhibit a fast increase in jasmonate levels in response to various stimuli and stresses, for example, wounding, herbivory, and infection. The widespread occurrence of jasmonates in plants and some lower eukaryotes and their capacity to regulate processes in insects support the notion that jasmonates are of general biological importance.[20]

Recently, it was reported that methyl jasmonate (**1**) exhibits anticancer activity in vitro and in vivo. The initial report indicated that methyl jasmonate induces suppression of cellular proliferation and death in various human and mouse cancer cell lines, including breast, prostate, melanoma, lymphoblastic leukemia, and lymphoma cells.[21] Furthermore, methyl jasmonate increased the life span of EL-4 lymphoma-bearing mice, and exhibited selective cytotoxicity towards cancer cells while sparing normal blood lymphocytes, even when the latter were part of a mixed population of leukemic and normal cells drawn from the blood of chronic lymphocytic leukemia (CLL) patients. Methyl jasmonate was also found to suppress the proliferation or kill various other cancer cells

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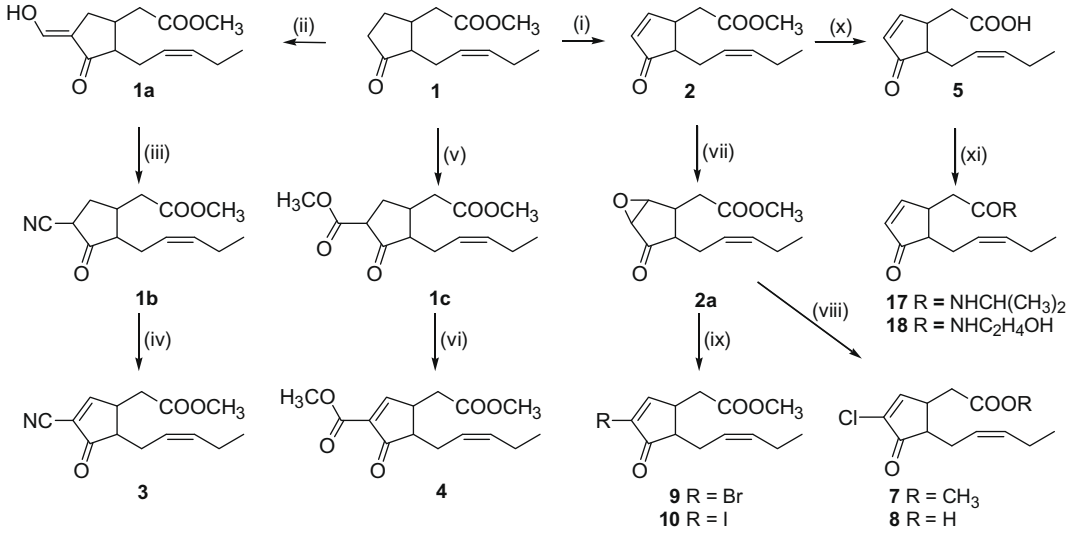
including lung and myeloid leukemia cells.[22–24] Thus, methyl jasmonate is now considered as a promising lead for cancer treatment in humans.[25–31] However, so far there was no report on the anti-inflammatory activity of this type of compound. Therefore, for our continuous search for new anti-inflammatory agents, the jasmonate skeleton was employed as a model structure for the synthesis of new cyclopentenone-bearing derivatives. In the present study, we report the synthesis and anti-inflammatory activity of jasmonate analogues.

2. Chemistry

The enone functionality of the aforementioned compounds, enone fatty acids,[1] sesquiterpene lactones (parthenolide),[2] cyclopentenone prostaglandins (PGA_1, PGA_2, and 15-deoxy-$\Delta^{12,14}$-PGJ_2),[3–12] and oleanane tritepenoids (CDDO),[13–16] is considered to be the key structure for their bioactivity. From a biochemical point of view, the enone group is an electrophilic center susceptible to nucleophilic attack (Michael addition) by a sulfhydryl group of reduced glutathione or cysteine residues in proteins. Alkylation of crucial cysteine residues can result in a loss of function[9] or, on the contrary, activation[4,10] of the target proteins. Therefore, introduction of an enone functionality is a critical step for design and synthesis of new jasmonate derivatives. Although many methods to introduce unsaturation adjacent to a carbonyl functionality have been developed over the years, the synthesis of α,β-unsaturated carbonyl compounds is often a tedious and sometimes challenging transformation.[32] Attempts to achieve one-step synthesis of **2** on the basis of the reported methods using several reagents, such as activated manganese dioxide (MnO_2),[33] benzeneselenic anhydride ($(PhSeO)_2O$),[34] and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) and *p*-toluenesulfonic acid (*p*-TsOH),[35] were unsatisfactory. In addition, synthesis of **2** via preparation of the silyl enol ether of **1** followed by palladium-catalyzed oxidation was also unsuccessful.[36] Recently, a new method for the synthesis of α,β-unsaturated carbonyl compounds in one simple step utilizing nontoxic IBX (*o*-iodoxybenzoic acid) as an oxidizing reagent has been reported.[37,38] However, the reaction was totally incomplete when tried in standard conditions. Therefore, modification of the reaction condition was attempted,[39] and the desired product **2** was obtained with a moderate yield by addition of a catalytic amount of TFA (trifluroacetic acid) at 80 °C for 48 h (Scheme 1).

Introduction of an electronegative group such as nitrile, carboxyl, or halogens at the α-position of the enone group is expected to increase the electrophilicity of the β-carbon, henceforth increasing bioactivity, as in the case of oleanane tritepenoids (CDDO)[13–16] and punaglandins.[40] To introduce an α-cyano enone group to **1**, formylation of **1** with ethyl formate in toluene in the presence of NaH and a catalytic amount of MeOH afforded the hydroxymethylene **1a**. Conversion of **1a** into a cyano group was performed according to a literature procedure,[41] using *O*,*N*-bis-(trifluoroacetyl)-hydroxylamine in a mixture of toluene and pyridine in reflux. This gave **1b** in 30% yield, and subsequent oxidation of **1b** with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in refluxing toluene afforded the required α-cyano enone **3**. Carboxylation of **1** with dimethyl carbonate in toluene in the presence of NaH and a catalytic amount of MeOH afforded the intermediate **1c** in 50% yield. Subsequent oxidation of **1c** with DDQ in refluxing toluene gave the α-methoxycarbonyl enone ester **4**. Attempts to obtain an α-carboxyl enone analogue by hydrolysis of **1c** were unfavorable and resulted in the reversion to **1**. The α-haloenone derivatives (**7**–**10**) were synthesized from **2** via two steps. The enone **2** was treated with alkaline H_2O_2 at −10 °C to generate the epoxide **2a**. The vinyl chlorides **7** and **8** were obtained concomitantly by treatment of the epoxide **2a** with HCl in acetic acid and $CHCl_3$.[42] However, the formation of vinyl bromide **9** was not obtained under the same condition (HBr/acetic acid/$CHCl_3$). Therefore, other conditions were tried, and the α-haloenone derivatives **9** and **10** were successfully prepared by treatment of **2a** with halide salts LiBr and LiI, respectively, under mildly acidic condition (silica gel support).[43]

In our previous study,[1] transformation of esters of enone fatty acids to corresponding acid or amide analogues was favorable to enhance anti-inflammatory activity of enone fatty acid derivatives. Therefore, this transformation of jasmonates is also expected to enhance potency. The acid analogue **5**, which was obtained by hydrolysis of **2** with porcine liver esterase in a mixture of acetone and phosphate buffer (pH 8), was the starting material for synthesis of **17** and **18**. Synthesis of amides from the acid **5** was tried using several coupling reagents, such as *N*,*N*-dicyclohexylcarbodiimide (DCC), 1-hydroxybenzotriazole (HOBt), or 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide HCl (DCCI).[44,45] However, the amides **17**

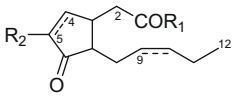


Scheme 1. Reagents and conditions: (i) IBX, TFA, DMSO, 80 °C, 48 h; (ii) NaH, MeOH (cat), ethyl formate, toluene, rt, 12 h; (iii) *O*,*N*-bis-(trifluoroacetyl)-hydroxylamine, pyridine, toluene, reflux, 1 h; (iv) DDQ, toluene, reflux, 10 min; (v) NaH, MeOH (cat), dimethyl carbonate, toluene, reflux, 3 h, then rt, 12 h; (vi) DDQ, toluene, reflux, 5 h; (vii) H_2O_2, 10% KOH, MeOH, 12 h; (viii) HCl/acetic acid, $CHCl_3$, 12 h; (ix) LiBr or LiI, silica gel, CH_2Cl_2, 24 h; (x) porcine liver esterase, phosphate buffer pH 8, acetone, rt, 12 h; (xi) TBTU, TEA, EtOAc, 24 h.

and **18** were only obtained by treatment of **5** with isopropylamine and ethanolamine, respectively, in the presence of 2-(1H-benzotriazole-1yl)-1,2,3,3-tetramethyluronium tetrafluoroborate (TBTU) and triethylamine (TEA) in ethyl acetate at room temperature.[46]

3. Results and discussion

Methyl jamonate (**1**) and its derivatives (**2**–**5** and **7**–**20**) were evaluated for their anti-inflammatory activity through measurement of their inhibitory effects on the production of pro-inflammatory mediators (NO, IL-6, and TNF-α) in murine macrophage cells (Figs. 1–3). As shown in Figure 1, the potency of compound **2** was significantly enhanced than the original compound (**1**), indicating the importance of an enone group for bioactivity of jasmonate derivatives. Also, compound **2** was more active than the α-cyano enone (**3**) and α-methoxycarbonyl enone (**4**), which is in contrast to the case of the α-cyano derivatives of oleanane triterpenoids (CDDO),[13–16] and this indicated that substitution of these groups at the α-position is detrimental to bioactivity. However, in the case of halogen substitution (such as Cl, Br, and I) at the α-position of the enone group, the activity was further enhanced than compound **2** (Fig. 2). In addition, the α-haloenones (**7**, **9**, **13**, and **15**) were much more potent than natural anti-inflammatory prostaglandins (PGA_1, PGA_2, and 15-deoxy-$\Delta^{12,14}$-PGJ_2) (Fig. 4).



1	$R_1 = OCH_3$	$R_2 = H$	$\Delta^{9}_{4,9}$
2	$R_1 = OCH_3$	$R_2 = H$	$\Delta^{9}_{4,9}$
3	$R_1 = OCH_3$	$R_2 = CN$	$\Delta^{9}_{4,9}$
4	$R_1 = OCH_3$	$R_2 = COOCH_3$	$\Delta^{9}_{4,9}$
5	$R_1 = OH$	$R_2 = H$	$\Delta^{9}_{4,9}$
7	$R_1 = OCH_3$	$R_2 = Cl$	$\Delta^{9}_{4,9}$
8	$R_1 = OH$	$R_2 = Cl$	$\Delta^{9}_{4,9}$
9	$R_1 = OCH_3$	$R_2 = Br$	$\Delta^{9}_{4,9}$
10	$R_1 = OCH_3$	$R_2 = I$	$\Delta^{9}_{4,9}$
11	$R_1 = OCH_3$	$R_2 = H$	Δ^{4}_{4}
12	$R_1 = OH$	$R_2 = H$	Δ^{4}_{4}
13	$R_1 = OCH_3$	$R_2 = Cl$	Δ^{4}_{4}
14	$R_1 = OH$	$R_2 = Cl$	Δ^{4}_{4}
15	$R_1 = OCH_3$	$R_2 = Br$	Δ^{4}_{4}
16	$R_1 = OCH_3$	$R_2 = I$	Δ^{4}_{4}
17	$R_1 = NHCH(CH_3)_2$	$R_2 = H$	$\Delta^{9}_{4,9}$
18	$R_1 = NHC_2H_4OH$	$R_2 = H$	$\Delta^{9}_{4,9}$
19	$R_1 = NHCH(CH_3)_2$	$R_2 = H$	Δ^{4}_{4}
20	$R_1 = NHC_2H_4OH$	$R_2 = H$	Δ^{4}_{4}

Regardless of other factors, the potency of α-haloenone derivatives was in the order Cl > Br > I, corresponding to the order of electronegativity. Their suppressive effects on the pro-inflammatory mediators were concentration-dependent (Figs. 2 and 3). At concentrations of 3.12 and 6.25 μM, compounds **9** and **15** showed the highest potency without any cytotoxicity (Fig. 3). However, at higher concentrations (12.5 and 25 μM), the brominated analogues (**9** and **15**) began to exhibit cytotoxicity to the cells (Figs. 2 and 3). The potency of the chlorinated analogues (**7** and **13**) was lower than the brominated derivatives (**9** and **15**); nonetheless, they would be safer because their cytotoxicity was negligible even at higher concentration (12.5 μM) (Fig. 3). Concerning the role of the methyl ester group, the ester forms (**2**, **7**, **11**, and **13**) were more active than the corresponding free acid (**5**, **8**, **12**, and **14**) or amide forms (**17**–**20**) (Fig. 2). Hence, in contrast to the activity of acid and amide derivatives observed for enone fatty acids in our previous study,[1] transformation of the ester

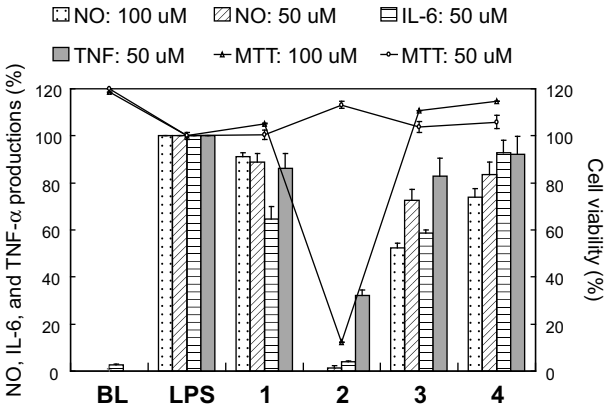


Figure 1. Inhibitory effects of methyl jasmonate (**1**) and its analogues (**2**–**4**) on productions of NO, IL-6, and TNF-α in LPS-activated RAW264.7 cells. RAW264.7 cells (1.5×10^5 cells/mL) were stimulated with LPS (1 μg/mL) alone or with test samples at concentrations of 50 and 100 μM for 24 h (BL, blank). The nitric oxide production was determined by Griess reagent method, and the production of pro-inflammatory cytokines (IL-6 and TNF-α) was determined by ELISA method. Cell viability was determined using MTT method. The data represent means ± SD of duplicate experiments.

group to other functional group such as acid (**5**, **8**, **12**, and **14**) or amide group (**17**–**20**) appeared to be unfavorable for anti-inflammatory activity of these analogues.

Regarding the influence of the unsaturation of the side chain on the activity, it was observed that compounds with a saturated side chain show higher potency, for example, **11** > **2**; **12** > **5**; **19** > **17**; **20** > **18**. However, this trend was inverted for α-haloenone derivatives, that is, derivatives with an unsaturated side chain showed higher potency, for example, **7** > **13**; **9** > **15**; **10** > **16** (Figs. 2 and 3). Taken together, the results revealed that the presence of an enone moiety is essential for the activity, but other structural modifications can dramatically modulate the activity of this type of compounds (Fig. 5).

Methyl jasmonate (**1**) contains two asymmetric centers at C-3 and C-7. Thus, this compound exists in four stereoisomeric forms, (3*R*,7*S*)- and (3*S*,7*R*)-*cis*-isomers, known as (+)- and (−)-epiMJA, respectively, and (3*R*,7*R*)- and (3*S*,7*S*)-*trans*-isomers, commonly called as (−)- and (+)-MJA, respectively (Fig. 6). The *cis*-isomers (epiMJAs) are less stable than *trans*-isomer (MJAs) due to higher steric hindrance between the *cis* side chains, and easily epimerized to the *trans*-isomers. The naturally occurring isomers in plants were reported to have the *R* stereochemistry at C-3 and either *S* or *R* at C-7.[47–49] The (3*R*,7*S*)-(+)-isomer, (+)-epiMJA, is believed to be the initial product formed in the biosynthesis of jasmonates in plants, and the (3*R*,7*R*)-(−)-isomer, (−)-MJA, is formed as a result of subsequent epimerization under physiological conditions in the cells or during isolation. Previous studies, in which the activities of pure stereoisomers of MJA were compared, revealed that (3*R*,7*S*)-(+)-epiMJA and (3*R*,7*R*)-(−)-MJA are the active isomers, while (3*S*,7*R*)-(−)-epiMJA and (3*S*,7*S*)-(+)-MJA are the inactive forms, and that the 3*R* configuration is indispensable for bioactivities of jasmonates.[50–54] Methyl jasmonate (**1**), a commercial product, was known to be an equilibrium mixture of approximately 45% each of (3*R*,7*R*)-(−)-MJA and (3*S*,7*S*)-(+)-MJA and 5% each of (3*R*,7*S*)-(+)-epiMJA and (3*S*,7*R*)-(−)-epiMJA.[52,54] Therefore, considering the consistent absolute configuration at the γ-carbon of the cyclopentenone moiety of natural anti-inflammatory prostaglandins (PGA_1, PGA_2, PGJ_2, and 15-deoxy-$\Delta^{12,14}$-PGJ_2) with inhibitory effects on the production and gene expression of pro-inflammatory mediators (NO, COX-2, IL-6, IL-1β, and TNF-α),[3–12] the enantiomer **2b** in the racemic mixture **2** would contribute most to the anti-inflammatory activity (Fig. 6).

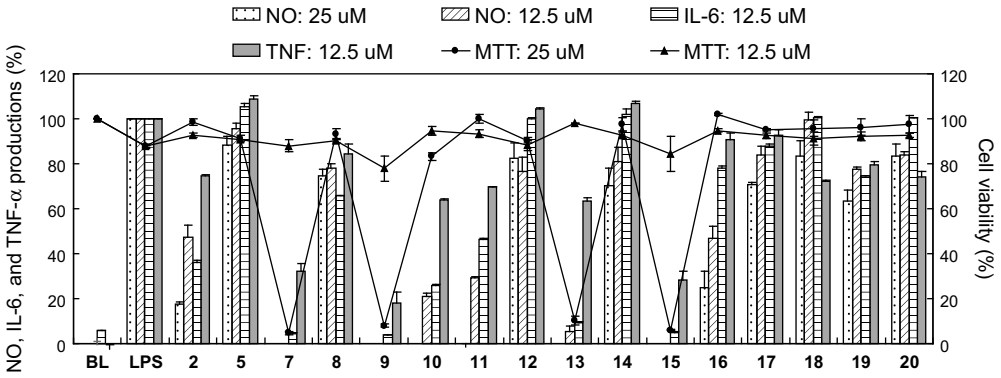


Figure 2. Inhibitory effects of jasmonate derivatives (**2**, **5**, and **7**–**20**) on productions of NO, IL-6, and TNF-α in LPS-activated RAW264.7 cells. RAW264.7 cells (1.5×10^5 cells/mL) were stimulated with LPS (1 μg/mL) alone or with test samples at concentrations of 12.5 and 25.0 μM for 24 h (BL, blank). The nitric oxide production was determined by Griess reagent method, and the production of pro-inflammatory cytokines (IL-6 and TNF-α) was determined by ELISA method. Cell viability was determined using MTT method. The data represent means ± SD of triplicate experiments.

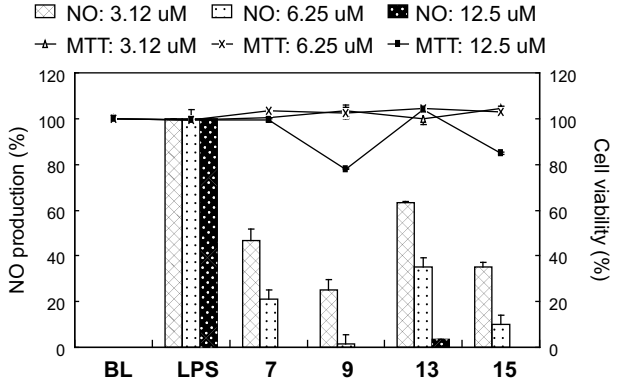


Figure 3. Inhibitory effects of compounds **7**, **9**, **13**, and **15** on the production of NO in LPS-activated RAW264.7 cells. RAW264.7 cells (1.5×10^5 cells/mL) were stimulated with LPS (1 μg/mL) alone or with test samples at concentrations of 3.12, 6.25, and 12.5 μM for 24 h (BL, blank). The nitric oxide production was determined by Griess reagent method. Cell viability was determined using MTT method. The data represent means ± SD of triplicate experiments.

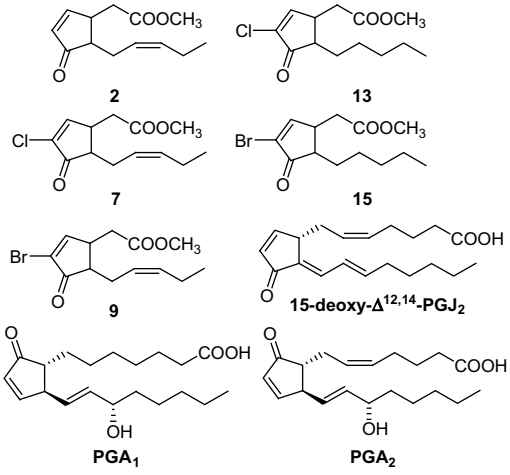
4. Conclusion

In this study, we have synthesized a series of new jasmonate derivatives and evaluated their anti-inflammatory activity. Of the synthesized analogues, the α-haloenones (**7**, **9**, **13**, and **15**) exhibited potent activity, and the activity of these compounds were much more enhanced than natural anti-inflammatory prostaglandins (PGA_1, PGA_2, and 15-deoxy-$\Delta^{12,14}$-PGJ_2). Among them, compounds **9** and **15** showed the highest potency, while compounds **7** and **13** would be more desirable with respect to safety. The results showed that jasmonate derivatives, especially α-haloenone jasmonates (**7**, **9**, **13**, and **15**), may serve as potential anti-inflammatory leads, and this is the first study demonstrating the anti-inflammatory potential of jasmonate analogues.

5. Experimental

5.1. General

^1H and ^{13}C spectra were recorded on Varian Inova 400 spectrometer. Chemical shifts were reported with reference to the respective residual solvent or deuterated solvent peaks (δ_H 7.24 and δ_C 77.0 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. Chemical reagents, including methyl jasmonate (**1**), PGA_1, PGA_2, and 15-deoxy-$\Delta^{12,14}$-PGJ_2, were purchased from the Sigma–Aldrich Chemical Company and used without further purification. Biological assays were performed at Cheju National University.



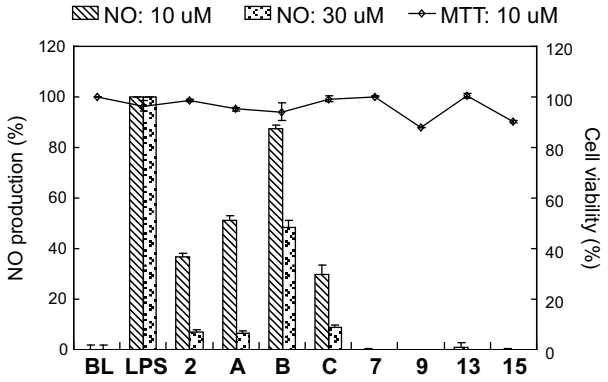


Figure 4. Inhibitory effects of compounds **2**, **7**, **9**, **13**, **15**, PGA_1 (A), PGA_2 (B), and 15-deoxy-$\Delta^{12,14}$-PGJ_2 (C) on the production of NO in LPS-activated RAW264.7 cells. RAW264.7 cells (1.5×10^5 cells/mL) were stimulated with LPS (1 μg/mL) alone or with test samples at concentrations of 10 and 30 μM for 24 h (BL, blank). The nitric oxide production was determined by Griess reagent method. Cell viability was determined using MTT method. The data represent means ± SD of triplicate experiments.

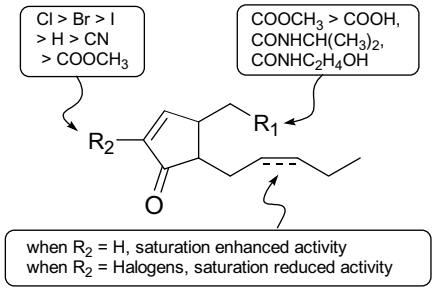


Figure 5. Structure–activity relationships of jasmonate derivatives. Anti-inflammatory activity was gauged as inhibition on the production of pro-inflammatory mediators (NO, IL-6, and TNF-α).

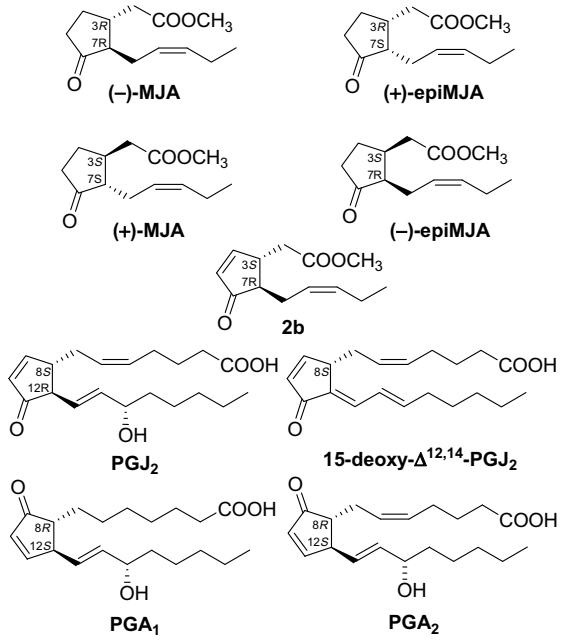


Figure 6. Stereochemistry of jasmonates and anti-inflammatory prostaglandins.

5.2. Synthesis of jasmonate analogues

5.2.1. Methyl 4,5-didehydrojasmonate (2)

To a stirred solution of methyl jasmonate **1** (50 mg) in DMSO (5 mL) were added IBX (*o*-iodoxybenzoic acid, 2.0 equiv) and trifluroacetic acid (0.3 equiv). The mixture was stirred at 80 °C for 48 h, and after cooling down, the reaction mixture was diluted with saturated $NaHCO_3$ solution and extracted with EtOAc (three times). The combined organic phase was washed with water and brine. After being dried over $MgSO_4$, the organic layer was concentrated. The resulting residue was purified by reversed-phase HPLC (YMC ODS-H80) eluting with 65% aqueous CH_3CN to yield compound **2** (15 mg, 30%); colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.61 (1H, dd, J = 6.0, 2.8 Hz, H-4), 6.17 (1H, dd, J = 6.0, 2.4 Hz, H-5), 5.46 (1H, m, H-10), 5.24 (1H, m, H-9), 3.70 (3H, s, OCH_3), 2.99 (1H, m, H-3), 2.57 (1H, dd, J = 15.6, 6.8 Hz, H-2b), 2.53–2.49 (1H, m, H-8b), 2.45 (1H, dd, J = 15.6, 8.4 Hz, H-2a), 2.29 (1H, m, H-8a), 2.06 (3H, m, H-7 and H-11), 0.94 (3H, t, J = 8.0 Hz, H-12); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 210.2 (C-6), 171.8 (C-1), 165.4 (C-4), 134.5 (C-10), 133.7 (C-5), 124.4 (C-9), 51.8 (OCH_3), 50.9 (C-7), 43.2 (C-3), 38.1 (C-2), 27.6 (C-8), 20.5 (C-11), 14.1 (C-12); FABMS m/z 223 $[M+H]^+$; HRFABMS m/z 223.1345 $[M+H]^+$.

5.2.2. Methyl 5-hydroxymethylene-jasmonate (1a)

To a stirred suspension of NaH (3.0 equiv) and a catalytic amount of MeOH in anhydrous toluene (50 mL) at 0 °C was added dropwise methyl jasmonate **1** (50 mg). After stirring at 0 °C for 10 min, ethyl formate (7.0 equiv) was added dropwise and the reaction mixture was stirred at room temperate overnight. Then, the mixture was extracted with cold water and the aqueous solution was re-extracted once with ether to remove any unreacted starting material. The aqueous solution was acidified with 10% HCl, and the orange-colored oily layer was removed by extraction with ether. This ethereal solution was washed with dilute $NaHCO_3$ to remove traces of excess acid and dried over $MgSO_4$. After evaporation of the ethereal solution, the residue (48 mg, 86%) obtained was used for the next reaction without purification; yellow oil; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.17 (1H, s), 5.47 (1H, m), 5.30 (1H, m), 3.68 (3H, s), 2.75 (1H, m), 2.62 (1H, m), 2.44–2.14 (6H, m), 2.03 (2H, m), 0.95 (3H, t, J = 7.6 Hz).

5.2.3. *O,N*-Bis-(trifluoroacetyl)-hydroxylamine

O,N-Bis-(trifluoroacetyl)-hydroxylamine was prepared by refluxing 3.2 moles of trifluoroacetic anhydride with 1 mole of hydroxylamine hydrochloride for 1.5 h. After removal of trifluoroacetic acid and trifluoroacetyl chloride under vacuum, the residue was recrystallized from CH_2Cl_2. The product is an 80% yield of hygroscopic and easily volatile needles of *O,N*-bis-(trifluoroacetyl)-hydroxylamine.[41]

5.2.4. Methyl 5-cyano-jasmonate (1b)

O,N-Bis-(trifluoroacetyl)-hydroxylamine (3.0 equiv) was added to a solution of **1a** (20 mg) and pyridine (6.0 equiv) in toluene and the resulting mixture was heated under reflux for 1 h. After cooling down, the organics were diluted with EtOAc, washed with H_2O, then brine, dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The crude product was purified by reversed-phase HPLC (YMC ODS-H80) eluting with 50% aqueous MeOH to yield compound **1b** (6.0 mg, 30%); colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 5.51 (1H, m), 5.21 (1H, m), 3.70 (3H, s), 3.40 (1H, dd, J = 8.4, 5.6 Hz, minor isomer), 3.10 (1H, dd, J = 12.8, 8.4 Hz, major isomer), 2.73 (1H, m), 2.56–2.29 (6H, m), 2.13 (1H, m), 2.03 (2H, m), 0.95 (3H, t, J = 7.2 Hz, minor isomer), 0.93 (3H, t, J = 7.2 Hz, major isomer).

5.2.5. Methyl 5-cyano-4,5-didehydrojasmonate (3)

A mixture of **1b** (5 mg) and 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ, 2.0 equiv) in anhydrous toluene was heated under reflux for 10 min. The insoluble matter was removed by filtration through a pipette plugged with cotton. The filtrate was concentrated in vacuo to give a residue, which was purified by reversed-phase HPLC (YMC ODS-H80) eluting with 70% aqueous MeOH to yield compound **3** (1.4 mg, 28%); colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.23 (1H, d, J = 2.4 Hz, H-4), 5.52 (1H, m, H-10), 5.19 (1H, m, H-9), 3.71 (3H, s, OCH_3), 3.13 (1H, m, H-3), 2.68 (1H, dd, J = 16.0, 6.0 Hz, H-2b), 2.54 (2H, m, H-2a and H-8b), 2.38 (1H, m, H-8a), 2.26 (1H, m, H-7), 2.02 (2H, m, H-11), 0.95 (3H, t, J = 7.6 Hz, H-12); FABMS m/z 248 $[M+H]^+$; HRFABMS m/z 248.1314 $[M+H]^+$.

5.2.6. Methyl 5-methoxycarbonyl-jasmonate (1c)

Methyl jasmonate **1** (20 mg) was added to a stirred suspension of NaH (2.5 equiv) and a catalytic amount of MeOH in toluene at room temperature. After stirring for 15 min, dimethyl carbonate (0.5 mL) was added and the reaction mixture was heated under reflux for 3 h. Then the reaction was stirred overnight, acidified with 10% HCl, and extracted with EtOAc (three times). The combined extracts were washed with water and brine, dried over $MgSO_4$, and filtered. The solvent was removed under reduced pressure and the crude product purified by reversed-phase HPLC (YMC ODS-H80) eluting with 70% aqueous CH_3CN to yield compound **1c** (12.5 mg, 50%); colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 5.45

(1H, m), 5.21 (1H, m), 3.74 (3H, s), 3.70 (3H, s), 3.34 (1H, dd, *J* = 9.6, 4.0 Hz, minor isomer), 3.13 (1H, dd, *J* = 12.4, 8.4 Hz, major isomer), 2.70 (1H, m), 2.59–2.18 (6H, m), 2.13–2.00 (3H, m), 0.94 (3H, t, *J* = 7.6 Hz).

5.2.7. Methyl 5-methoxycarbonyl-4,5-didehydrojasmonate (4)

A mixture of **1c** (5 mg) and 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ, 2.0 equiv) in anhydrous toluene was heated under reflux for 5 h. The insoluble matter was removed by filtration through a pipette plugged with cotton. The filtrate was concentrated in vacuo to give a residue, which was purified by reversed-phase HPLC (YMC ODS-H80) eluting with 50% aqueous CH_3CN to yield compound **4** (1.5 mg, 30%); colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.31 (1H, d, *J* = 2.4 Hz, H-4), 5.48 (1H, m, H-10), 5.22 (1H, m, H-9), 3.82 (3H, s, OCH_3), 3.71 (3H, s, OCH_3), 3.13 (1H, m, H-3), 2.64(1H, dd, *J* = 16.0, 6.0 Hz, H-2b), 2.58–2.51 (1H, m, H-8b), 2.49 (1H, dd, *J* = 16.0, 8.4 Hz, H-2a), 2.35 (1H, m, H-8a), 2.25 (1H, m, H-7), 2.04 (2H, m, H-11), 0.94 (3H, t, *J* = 7.6 Hz, H-12); FABMS *m/z* 281 $[M+H]^+$; HRFABMS *m/z* 281.1400 $[M+H]^+$.

5.2.8. 4,5-Didehydrojasmonic acid (5)

To a solution of compound **2** (8 mg, 36 μM) in acetone (0.1 mL) were added phosphate buffer (pH 8.0, 0.9 mL) and 12 μL (36 U) porcine liver esterase (Sigma Chemical; Lot No. E2884-1KU; 0.34 mL, 16.5 mg protein/mL, and 177 U/mg protein) at ambient temperature, and the mixture was stirred overnight. Then the solution was acidified with diluted HCl solution to pH 4, and the mixture was extracted with EtOAc. The organic extracts were dried over $MgSO_4$, filtered, and evaporated to give a residue, which was purified by reversed-phase HPLC (YMC ODS-H80) eluting with 65% aqueous CH_3CN to yield compound **5** (6 mg, 80%); colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.63 (1H, dd, *J* = 6.0, 2.4 Hz, H-4), 6.20 (1H, dd, *J* = 6.0, 2.0 Hz, H-5), 5.45 (1H, m, H-10), 5.23 (1H, m, H-9), 2.99 (1H, m, H-3), 2.63 (1H, dd, *J* = 15.6, 6.8 Hz, H-2b), 2.55–2.50 (1H, m, H-8b), 2.49 (1H, dd, *J* = 15.6, 8.4 Hz, H-2a), 2.30 (1H, m, H-8a), 2.09 (1H, m, H-7), 2.03 (2H, m, H-11), 0.93 (3H, t, *J* = 7.2 Hz, H-12); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 210.3 (C-6), 177.0 (C-1), 165. 2 (C-4), 134.6 (C-10), 133.9 (C-5), 124.2 (C-9), 50.9 (C-7), 42.9 (C-3), 37.9 (C-2), 27.6 (C-8), 20.5 (C-11), 14.1 (C-12); FABMS *m/z* 209 $[M+H]^+$; HRFABMS *m/z* 209.1174 $[M+H]^+$.

5.2.9. Methyl 5-chloro-4,5-didehydrojasmonate (7)

To a stirred methanolic solution of **2** (20 mg) at −10 °C was added a solution of 30% hydrogen peroxide (50 μL) in one portion. To the above mixture was added dropwise 10% KOH (12 μL) and kept stirring at this temperature for 12 h. The reaction mixture was neutralized with 1 N HCl and concentrated by evaporating off methanol. The residual oil was dissolved in EtOAc, washed with water, dried over MgSO4, and concentrated to dryness in vacuo to give the epoxide **2a** (20 mg), which was used for the next reaction without purification. A solution of **2a** (5 mg) in acetic acid including 1 M HCl and $CHCl_3$ was stirred at room temperature for 12 h. The mixture was diluted with CH_2Cl_2 and washed with water three times. After working up according to the standard method, the residue was purified by using reversed-phase HPLC (YMC ODS-H80) eluting with 65% aqueous CH_3CN to yield compound **7** (2.0 mg, 38%); colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.52 (1H, d, *J* = 2.4 Hz, H-4), 5.48 (1H, m, H-10), 5.21 (1H, m, H-9), 3.70 (3H, s, OCH_3), 2.96 (1H, m, H-3), 2.60 (1H, dd, *J* = 16.0, 6.4 Hz, H-2b), 2.56–2.51 (1H, m, H-8b), 2.45 (1H, dd, *J* = 16.0, 7.6 Hz, H-2a), 2.35 (1H, m, H-8a), 2.22 (1H, m, H-7), 2.04 (2H, m, H-11), 0.94 (3H, t, *J* = 7.6 Hz, H-12); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 201.4 (C-6), 171.4 (C-1), 158.1 (C-4), 135.7 (C-5), 135.2 (C-10), 123.6 (C-9), 52.0 (OCH_3), 50.2 (C-7), 40.4 (C-3), 37.9 (C-2), 27.8 (C-8), 20.6 (C-11), 14.1 (C-12); FABMS *m/z* 257 $[M+H]^+$; HRFABMS *m/z* 257.0937 $[M+H]^+$.

5.2.10. 5-Chloro-4,5-didehydrojasmonic acid (8)

Compound **8** (0.8 mg, 16%) was formed concomitantly with compound **7**; colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.54 (1H, d, *J* = 2.8 Hz, H-4), 5.50 (1H, m, H-10), 5.23 (1H, m, H-9), 2.98 (1H, m, H-3), 2.68 (1H, dd, *J* = 16.0, 6.4 Hz, H-2b), 2.59–2.52 (1H, m, H-8b), 2.50 (1H, dd, *J* = 16.0, 7.6 Hz, H-2a), 2.37 (1H, m, H-8a), 2.25 (1H, m, H-7), 2.06 (2H, m, H-11), 0.94 (3H, t, *J* = 7.6 Hz, H-12); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 201.5 (C-6), 176.6 (C-1), 157.9 (C-4), 135.9 (C-5), 135.3 (C-10), 123.2 (C-9), 50.2 (C-7), 40.1 (C-3), 37.7 (C-2), 27.8 (C-8), 20.6 (C-11), 14.1 (C-12); FABMS *m/z* 243 $[M+H]^+$; HRFABMS *m/z* 243.0765 $[M+H]^+$.

5.2.11. Methyl 5-bromo-4,5-didehydrojasmonate (9)

To a mixture of compound **2a** (5 mg) and LiBr (3 equiv) in a few mL of CH_2Cl_2 was added silica gel (200 mg/mmol). The suspension was shaken at room temperature for a while and evaporated to dryness. After allowing to stand at this temperature for 24 h, silica gel was removed by filtration and the crude product was purified by reversed-phase HPLC (YMC ODS-H80) eluting with 65% aqueous CH_3CN to yield compound **9** (1.0 mg, 16%); yellow oil; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.72 (1H, d, *J* = 2.8 Hz, H-4), 5.45 (1H, m, H-10), 5.23 (1H, m, H-9), 3.70 (3H, s, OCH_3), 2.99 (1H, m, H-3), 2.60 (1H, dd, *J* = 16.0, 6.0 Hz, H-2b), 2.56–2.51 (1H, m, H-8b), 2.45 (1H, dd, *J* = 16.0, 8.4 Hz, H-2a), 2.36 (1H, m, H-8a), 2.22 (1H, m, H-7), 2.03 (2H, m, H-11), 0.94 (3H, t, *J* = 7.6 Hz, H-12); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 202.0 (C-6), 171.4 (C-1), 162.6 (C-4), 135.2 (C-10), 125.8 (C-5), 123.6 (C-9), 51.9 (OCH_3), 49.8 (C-7), 42.4 (C-3), 37.8 (C-2), 27.9 (C-8), 20.6 (C-11), 14.1 (C-12); FABMS *m/z* 301/303 $[M+H]^+$.

5.2.12. Methyl 5-iodo-4,5-didehydrojasmonate (10)

Compound **2a** (5 mg), LiI (3 equiv), and silica gel (200 mg/mmol) in a few mL of CH_2Cl_2 were treated as in the method for preparation of compound **9** to afford **10** (1.5 mg, 21%); colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.96 (1H, d, *J* = 2.4 Hz, H-4), 5.48 (1H, m, H-10), 5.20 (1H, m, H-9), 3.70 (3H, s, OCH_3), 3.03 (1H, m, H-3), 2.58 (1H, dd, *J* = 16.0, 6.4 Hz, H-2b), 2.54–2.50 (1H, m, H-8b), 2.45 (1H, dd, *J* = 16.0, 8.8 Hz, H-2a), 2.34 (1H, m, H-8a), 2.19 (1H, m, H-7), 2.04 (2H, m, H-11), 0.94 (3H, t, *J* = 7.6 Hz, H-12); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 204.0 (C-6), 171.4 (C-1), 170.3 (C-4), 135.1 (C-10), 123.7 (C-9), 102.6 (C-5), 51.9 (OCH_3), 48.5 (C-7), 45.3 (C-3), 37.8 (C-2), 27.9 (C-8), 20.6 (C-11), 14.1 (C-12); FABMS *m/z* 349 $[M+H]^+$; HRFABMS *m/z* 349.0314 $[M+H]^+$.

5.2.13. Methyl 4,5-didehydro-dihydrojasmonate (11)

Methyl dihydrojasmonate (50 mg), which was obtained by hydrogenation of **1** under palladium–charcoal (10% Pd–C) catalysis, IBX (2 equiv), and TFA (0.3 equiv) in DMSO were treated as in the method for preparation of compound **2** to afford **11** (16 mg, 32%); colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.60 (1H, dd, *J* = 5.6, 2.4 Hz, H-4), 6.14 (1H, dd, *J* = 5.6, 2.4 Hz, H-5), 3.70 (3H, s, OCH_3), 3.00 (1H, m, H-3), 2.56 (1H, dd, *J* = 16.0, 7.2 Hz, H-2b), 2.44 (1H, dd, *J* = 16.0, 8.4 Hz, H-2a), 1.98 (1H, m, H-7), 1.70 (1H, m, H-8b), 1.45 (1H, m, H-8a), 1.29 (6H, m, H-9, H-10, and H-11), 0.85 (3H, t, *J* = 6.4 Hz, H-12); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 211.0 (C-6), 171.9 (C-1), 165.1 (C-4), 133.7 (C-5), 51.8 (OCH_3), 51.2 (C-7), 43.9 (C-3), 38.4 (C-2), 31.9 (C-10), 30.7 (C-8), 26.5 (C-9), 22.4 (C-11), 14.0 (C-12); FABMS *m/z* 225 $[M+H]^+$; HRFABMS *m/z* 225.1500 $[M+H]^+$.

5.2.14. 4,5-Didehydro-dihydrojasmonic acid (12)

Compound **11** (8 mg) and porcine liver esterase (12 μL) in a mixture of acetone (0.1 mL) and phosphate buffer (pH 8.0, 0.9 mL) were treated as in the method for preparation of compound **5** to afford **12** (6 mg, 80%); colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.64 (1H, dd, *J* = 5.2, 2.4 Hz, H-4), 6.18 (1H, dd,

J = 5.2, 2.4 Hz, H-5), 3.02 (1H, m, H-3), 2.63 (1H, dd, J = 16.0, 6.4 Hz, H-2b), 2.49 (1H, dd, J = 16.0, 8.8 Hz, H-2a), 2.03 (1H, m, H-7), 1.73 (1H, m, H-8b), 1.47 (1H, m, H-8a), 1.30 (6H, m, H-9, H-10, and H-11), 0.85 (3H, t, J = 6.8 Hz, H-12); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 211.1 (C-6), 177.1 (C-1), 165.0 (C-4), 133.9 (C-5), 51.2 (C-7), 43.6 (C-3), 38.2 (C-2), 31.9 (C-10), 30.7 (C-8), 26.5 (C-9), 22.4 (C-11), 14.0 (C-12); FABMS m/z 211 $[M+H]^+$; HRFABMS m/z 211.1349 $[M+H]^+$.

5.2.15. Methyl 5-chloro-4,5-didehydro-dihydrojasmonate (13)

Compound **11a** (5 mg), which was obtained by epoxidation of **11**, in acetic acid including 1 M HCl and $CHCl_3$ were treated as in the method for preparation of compound **7** to afford **13** (2.1 mg, 40%); colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.53 (1H, d, J = 2.4 Hz, H-4), 3.71 (3H, s, OCH_3), 2.99 (1H, m, H-3), 2.60 (1H, dd, J = 16.0, 6.0 Hz, H-2b), 2.46 (1H, dd, J = 16.0, 8.8 Hz, H-2a), 2.14 (1H, m, H-7), 1.75 (1H, m, H-8b), 1.53 (1H, m, H-8a), 1.29 (6H, m, H-9, H-10, and H-11), 0.86 (3H, t, J = 6.8 Hz, H-12); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 202.0 (C-6), 171.5 (C-1), 157.9 (C-4), 135.7 (C-5), 52.0 (OCH_3), 50.4 (C-7), 41.2 (C-3), 38.2 (C-2), 31.8 (C-10), 30.7 (C-8), 26.3 (C-9), 22.4 (C-11), 14.0 (C-12); FABMS m/z 259 $[M+H]^+$; HRFABMS m/z 259.1103 $[M+H]^+$.

5.2.16. 5-Chloro-4,5-didehydro-dihydrojasmonic acid (14)

Compound **14** (0.7 mg, 14%) was formed concomitantly with compound **13**; colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.55 (1H, d, J = 2.4 Hz, H-4), 2.99 (1H, m, H-3), 2.68 (1H, dd, J = 16.4, 6.0 Hz, H-2b), 2.51 (1H, dd, J = 16.4, 9.6 Hz, H-2a), 2.17 (1H, m, H-7), 1.76 (1H, m, H-8b), 1.55 (1H, m, H-8a), 1.31 (6H, m, H-9, H-10, and H-11), 0.87 (3H, t, J = 6.8 Hz, H-12); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 202.1 (C-6), 176.7 (C-1), 157.7 (C-4), 135.5 (C-5), 50.4 (C-7), 40.9 (C-3), 38.0 (C-2), 31.8 (C-10), 30.7 (C-8), 26.3 (C-9), 22.4 (C-11), 14.0 (C-12); FABMS m/z 245 $[M+H]^+$; HRFABMS m/z 245.0961 $[M+H]^+$.

5.2.17. Methyl 5-bromo-4,5-didehydro-dihydrojasmonate (15)

Compound **11a** (5 mg), LiBr (3 equiv), and silica gel (200 mg/mmol) in a few mL of CH_2Cl_2 were treated as in the method for preparation of compound **9** to afford **15** (1.1 mg, 18%); yellow oil; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.73 (1H, d, J = 2.8 Hz, H-4), 3.72 (3H, s, OCH_3), 2.99 (1H, m, H-3), 2.60 (1H, dd, J = 16.0, 6.4 Hz, H-2b), 2.46 (1H, dd, J = 16.0, 8.8 Hz, H-2a), 2.14 (1H, m, H-7), 1.76 (1H, m, H-8b), 1.50 (1H, m, H-8a), 1.29 (6H, m, H-9, H-10, and H-11), 0.86 (3H, t, J = 6.8 Hz, H-12); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 202.0 (C-6), 171.4 (C-1), 162.3 (C-4), 125.6 (C-5), 52.0 (OCH_3), 50.0 (C-7), 43.2 (C-3), 38.0 (C-2), 31.8 (C-10), 30.9 (C-8), 26.3 (C-9), 22.4 (C-11), 14.0 (C-12); FABMS m/z 303/305 $[M+H]^+$.

5.2.18. Methyl 5-iodo-4,5-didehydro-dihydrojasmonate (16)

Compound **11a** (5 mg), LiI (3 equiv), and silica gel (200 mg/mmol) in a few mL of CH_2Cl_2 were treated as in the method for preparation of compound **9** to afford **16** (2.1 mg, 30%); colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.97 (1H, d, J = 2.4 Hz, H-4), 3.71 (3H, s, OCH_3), 3.05 (1H, m, H-3), 2.58 (1H, dd, J = 15.6, 5.6 Hz, H-2b), 2.45 (1H, dd, J = 15.6, 8.8 Hz, H-2a), 2.14 (1H, m, H-7), 1.73 (1H, m, H-8b), 1.55 (1H, m, H-8a), 1.28 (6H, m, H-9, H-10, and H-11), 0.86 (3H, t, J = 6.8 Hz, H-12); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 204.0 (C-6), 171.4 (C-1), 170.0 (C-4), 102.7 (C-5), 52.0 (OCH_3), 48.7 (C-7), 46.1 (C-3), 38.0 (C-2), 31.8 (C-10), 30.9 (C-8), 26.3 (C-9), 22.4 (C-11), 14.0 (C-12); FABMS m/z 351 $[M+H]^+$; HRFABMS m/z 351.0450 $[M+H]^+$.

5.2.19. *N*-Isopropyl-4,5-didehydrojasmonylamide (17)

To a mixture of compound **5** (2 mg) in EtOAc and triethylamine (TEA, 2 equiv), was added 2-(1H-benzotriazole-1yl)-1,2,3,3-tetramethyluronium tetrafluoroborate (TBTU, 1 equiv). After stirring for 1 h at room temperature, isopropylamine (2 equiv) was added and the reaction mixture was stirred for 12 h. The mixture was washed with water, dried, and concentrated to give a residue which was purified by reversed-phase HPLC (YMC ODS-H80) eluting with 65% aqueous CH_3CN to yield compound **17** (1.5 mg, 63%); colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.65 (1H, dd, J = 5.6, 2.8 Hz, H-4), 6.14 (1H, dd, J = 5.6, 2.4 Hz, H-5), 5.45 (1H, m, H-10), 5.26 (1H, m, H-9), 4.07 (1H, septet, J = 6.4 Hz, $-CH(CH_3)_2$), 3.08 (1H, m, H-3), 2.50 (1H, m, H-8b), 2.35 (1H, dd, J = 14.4, 6.8 Hz, H-2b), 2.29 (1H, m, H-8a), 2.22 (1H, dd, J = 14.4, 8.4 Hz, H-2a), 2.05 (3H, m, H-7 and H-11), 1.14 (6H, d, J = 6.8 Hz, $-(CH_3)_2$), 0.94 (3H, t, J = 7.2 Hz, H-12); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 210.6 (C-6), 169.3 (C-1), 166.4 (C-4), 134.4 (C-10), 133.4 (C-5), 124.5 (C-9), 51.1 (C-7), 43.9 (C-3), 41.5 (C-2), 40.9 ($-CH(CH_3)_2$), 27.8 (C-8), 22.8 ($-(CH_3)_2$), 20.6 (C-11), 14.2 (C-12); FABMS m/z 250 $[M+H]^+$; HRFABMS m/z 250.1798 $[M+H]^+$.

5.2.20. *N*-Hydroxyethyl-4,5-didehydrojasmonylamide (18)

Compound **5** (2 mg), TBTU (1 equiv), TEA (2 equiv), and ethanolamine (2 equiv) in EtOAc were treated as in the method for preparation of compound **17** to afford **18** (1.3 mg, 54%); yellow oil; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.66 (1H, dd, J = 5.6, 2.4 Hz, H-4), 6.15 (1H, dd, J = 5.6, 2.0 Hz, H-5), 5.89 (1H, br s, NH), 5.46 (1H, m, H-10), 5.26 (1H, m, H-9), 3.73 (2H, t, J = 5.6 Hz, $-CH_2CH_2OH$), 3.44 (2H, quint, J = 6.0 Hz, $-CH_2OH$), 3.08 (1H, m, H-3), 2.50 (1H, m, H-8b), 2.46 (1H, dd, J = 14.8, 6.8 Hz, H-2b), 2.30 (2H, m, H-2a and H-8a), 2.06 (3H, m, H-7 and H-11), 0.94 (3H, t, J = 7.6 Hz, H-12); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 210.6 (C-6), 171.3 (C-1), 166.2 (C-4), 134.5 (C-10), 133.5 (C-5), 124.5 (C-9), 62.2 ($-CH_2OH$), 51.1 (C-7), 43.8 (C-3), 42.2(C-2), 40.5 ($-CH_2CH_2OH$), 27.8 (C-8), 20.6 (C-11), 14.2 (C-12); FABMS m/z 252 $[M+H]^+$; HRFABMS m/z 252.1588 $[M+H]^+$.

5.2.21. *N*-Isopropyl-4,5-didehydro-dihydrojasmonylamide (19)

Compound **12** (2 mg), TBTU (1 equiv), TEA (2 equiv), and isopropylamine (2 equiv) in EtOAc were treated as in the method for preparation of compound **17** to afford **19** (1.5 mg, 63%); colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.63 (1H, dd, J = 5.2, 2.4 Hz, H-4), 6.12 (1H, dd, J = 5.2, 1.6 Hz, H-5), 5.29 (1H, br s, NH), 4.08 (1H, septet, J = 6.8 Hz, $-CH(CH_3)_2$), 3.11 (1H, m, H-3), 2.33 (1H, dd, J = 14.0, 6.8 Hz, H-2b), 2.22 (1H, dd, J = 14.0, 8.4 Hz, H-2a), 1.93 (1H, m, H-7), 1.68 (1H, m, H-8b), 1.47 (1H, m, H-8a), 1.30 (6H, m, H-9, H-10, and H-11), 1.15 (6H, d, J = 6.8 Hz, $-(CH_3)_2$), 0.85 (3H, t, J = 6.4 Hz, H-12); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 211.5 (C-6), 169.3 (C-1), 166.1 (C-4), 133.3 (C-5), 51.4 (C-7), 44.5 (C-3), 41.5 (C-2), 41.2 ($-CH(CH_3)_2$), 31.9 (C-10), 30.8 (C-8), 26.5 (C-9), 22.8 ($-(CH_3)_2$), 22.4 (C-11), 14.0 (C-12); FABMS m/z 252 $[M+H]^+$; HRFABMS m/z 252.1945 $[M+H]^+$.

5.2.22. *N*-Hydroxyethyl-4,5-didehydro-dihydrojasmonylamide (20)

Compound **12** (2 mg), TBTU (1 equiv), TEA (2 equiv), and ethanolamine (2 equiv) in EtOAc were treated as in the method for preparation of compound **17** to afford **20** (1.2 mg, 50%); yellow oil; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.65 (1H, dd, J = 6.0, 2.4 Hz, H-4), 6.13 (1H, dd, J = 6.0, 2.0 Hz, H-5), 5.94 (1H, br s, NH), 3.73 (2H, t, J = 5.2 Hz, $-CH_2CH_2OH$), 3.58 (2H, quint, J = 6.0 Hz, $-CH_2OH$), 3.11 (1H, m, H-3), 2.44 (1H, dd, J = 14.8, 6.8 Hz, H-2b), 2.30 (1H, dd, J = 14.8, 8.4 Hz, H-2a), 1.96 (1H, m, H-7), 1.70 (1H, m, H-8b), 1.48 (1H, m, H-8a), 1.30 (6H, m, H-9, H-10, and H-11), 0.85 (3H, t, J = 6.8 Hz, H-12); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 211.4 (C-6), 171.4 (C-1), 165.9 (C-4), 133.6 (C-5), 62.2 ($-CH_2OH$), 51.4 (C-7), 44.4 (C-3), 42.2 (C-2), 40.9

(–CH_2CH_2OH), 31.9 (C-10), 30.8 (C-8), 26.5 (C-9), 22.5 (C-11), 14.0 (C-12); FABMS *m/z* 254 $[M+H]^+$; HRFABMS *m/z* 254.1763 $[M+H]^+$.

5.3. Cell culture

The murine 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.

5.4. Cytotoxicity assay

Cytotoxic effects were evaluated in cells cultured for 24 h using the MTT (3-[4,5-dimethylthiazol-2yl]-2,5-diphenyl tetrazolium bromide) assay. MTT was added to cells and, after 4 h, cultures were removed from the incubator and the formazan crystals were dissolved by adding DMSO. Metabolic activity was quantified by measuring light absorbance at 540 nm.

5.5. Nitrite assay

The production of nitric oxide (NO) was measured, as previously described by Ryu et al.,[55] by using the Griess reagent (Sigma, MO, USA). 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% naphthylethylenediamine 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 ELISA reader (Amersham Pharmacia Biotech, UK, USA) and the results were compared against a calibration curve using sodium nitrite as the standard.

5.6. Pro-inflammatory cytokines (IL-6 and TNF-α) production

The inhibitory effects of jasmonate analogues on IL-6 and TNF-α production were determined by the method previously described.[56] The samples were solubilized with EtOH diluted with DMEM. The final concentration of chemical solvents should not exceed 0.1% in the culture medium. At these conditions, none of the 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 kit (R&D Systems Inc., MN, USA).

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