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Evaluation of endogenous fatty acid amides and their synthetic analogues as potential anti-inflammatory leads

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ABSTRACT

A series of endogenous fatty acid amides and their analogues (**1**–**78**) were prepared, and their inhibitory effects on pro-inflammatory mediators (NO, IL-1β, IL-6, and TNF-α) in LPS-activated RAW264.7 cells were evaluated. Their inhibitory activity on the pro-inflammatory chemokine MDC in IFN-γ-activated HaCaT cells was also examined. The results showed that the activity is strongly dependent on the nature of the fatty acid part of the molecules. As expected, the amides derived from enone fatty acids showed significant activity and were more active than those derived from other types of fatty acids. A variation of the amine headgroup also altered bioactivity profile remarkably, possibly by modulating cell permeability. Regarding the amine part of the molecules, *N*-acyl dopamines exhibited the most potent activity (IC_{50} ~2 μM). This is the first report of the inhibitory activity of endogenous fatty acid amides and their analogues on the production of nitric oxide, cytokines (IL-1β, IL-6, and TNF-α) and the chemokine MDC. This study suggests that the enone fatty acid-derived amides (such as *N*-acyl ethanolamines and *N*-acyl amino acids) and *N*-acyl dopamines may be potential anti-inflammatory leads.

1. Introduction

As part of an ongoing search for new anti-inflammatory leads targeting nitric oxide (NO) and cytokines (IL-1β, IL-6, and TNF-α) that play important roles in the inflammatory process and the immune responses,[1–3] this study examined the derivatization of new C_{18} enone fatty acids isolated from the red alga *Gracilaria verrucosa* via bioassay guidance.[4] Although these compounds had simple structures, they exhibited interesting biological activities. The C_{18} enone fatty acids exhibited anti-inflammatory activity by inhibiting the production of pro-inflammatory mediators (NO, IL-1β, IL-6, and TNF-α) through the down-regulation of NF-κB and STAT1 activity in LPS-activated RAW264.7 cells.[5] These compounds also exhibited significant apoptotic activity[6] and anti-angiogenic activity (data not published). Based on previous studies, it was speculated that the α,β-unsaturated ketone (enone) functionality, which has been encountered in many potential anti-inflammatory compounds such as curcumin,[7] sesquiterpene lactones (parthenolide),[8] cyclopentenone prostaglandins (PGA_1, PGA_2, and 15-deoxy-$\Delta^{12,14}$-PGJ_2)[9] and oleanane tritepenoids (CDDO-Me or Bardoxolone),[10] is essential for the activity of these unusual fatty acids. Quite recently, another new class of C_{22} enone fatty acids, namely EFOX (electrophilic oxo derivatives) derived from ω-3 fatty acids docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA), was reported.[11] These C_{22} enone fatty acids are generated during inflammation via a COX-2-catalyzed mechanism and act as anti-inflammatory mediators. By interacting with certain nucleophilic protein residues, these enone fatty acids induced changes in cellular protein function and gene expression, resulting in a wide range of anti-oxidant and anti-inflammatory responses. Accordingly, these electrophilic fatty acids can serve as PPAR-γ agonists and inhibit pro-inflammatory cytokines and NO production within biological concentration ranges. The discovery of this new class of C_{22} enone fatty acids as anti-inflammatory mediators explained the beneficial clinical effects of ω-3 fatty acids.[11] These observations along with our findings suggest that synthesis of C_{18} enone fatty acid analogues that can mimic the action of natural anti-inflammatory mediators derived from ω-3 fatty acids might provide potential inflammation modulators. Therefore, considering the preliminary data on the structure-activity relationship (SAR) of C_{18} enone fatty acids,[4] the transformation of these new fatty acids into amide counterparts is expected to produce potential anti-inflammatory leads.

Our design of anti-inflammatory fatty acid amides was inspired by a class of endogenous lipid mediators, such as anandamide.[12–14]

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These endogenous fatty acid amides are a large family of structurally diverse molecules found in mammalian systems. Many are comprised of a long chain fatty acid and a more polar group, such as ammonia (primary fatty acid amides), ethanolamine (*N*-acyl ethanolamines), dopamine (*N*-acyl dopamines) or amino acids (*N*-acyl amino acids or lipoamino acids). The fatty acid component of the structure can range from fully saturated, such as palmitic acid, to a highly unsaturated, such as arachidonic acid. Included in the list of endogenous fatty acid amides are the widely studied *N*-arachidonoyl ethanolamide (AEA, anandamide), *N*-oleyl ethanolamide (OEA) and *N*-palmitoyl ethanolamide (PEA). Owing to its actions on the cannabinoid (CB-1) and vanilloid receptors (VR-1), AEA possesses a number of interesting pharmacological properties including effects on nociception, memory processes, lung function, spasticity, appetite, cell proliferation, immune response and inflammatory process. PEA also exhibits a range of activities including analgesic, anti-inflammatory and anticonvulsant properties despite the lack of cannabinoid receptor affinity. In contrast to AEA that causes overeating, OEA decreases the level of food intake and body weight by activating PPAR-α but independent of the cannabinoid receptors. However, the half-life of all these endogenous fatty acids amides is very short due to rapid deactivation by fatty acid amide hydrolase (FAAH).[12–14]

Owing to the broad spectrum of biological activities exhibited by endogenous fatty acid amide family, a wide range of indications, including cancer, cardiovascular disease, inflammation, pain, drug addiction, eating disorders, anxiety and depression, may benefit from fatty acid amide-derived agents. Potential drug targets include the enzymes involved in endogenous fatty acid amide degradation and transporters responsible for moving them across the cell membrane. On the other hand, analogues of endogenous fatty acid amides may serve as agonists or antagonists for their respective receptors.[12,15] Accordingly, considerable research has focused on the search for new fatty acid amide derivatives for the development of novel analgesic agents, targeting mainly FAAH, CB-1, and VR-1.[16] However, little attention has been paid to inhibitors targeting NO and cytokines (IL-1β, IL-6, and TNF-α), which play a key role in the inflammatory process and immune responses, even though the anti-inflammatory and immunomodulatory activities of some members from this family have been mentioned.[12–14] In addition, little is known regarding the effects of endogenous fatty acid amides on these inflammatory mediators, despite the increasing awareness that these compounds and their related analogues also exerted biological activities through cannabinoid CB-1 and vanilloid VR-1 receptors-independent pathways.[17] Therefore, an examination of the inhibitory effects of endogenous fatty acid amides and their analogues on pro-inflammatory mediators (NO, IL-1β, IL-6, and TNF-α) might further extend our knowledge on the therapeutic anti-inflammatory efficacy of this class of compound and help search for potential anti-inflammatory leads.

In view of this background, the replacement of the fatty acid component of endogenous fatty acid amides with the C_{18} enone fatty acid skeleton is expected to provide better anti-inflammatory leads. Therefore, endogenous fatty acid amides and their analogues (**1**–**78**) were prepared, and their inhibitory effects on pro-inflammatory mediators (NO, IL-1β, IL-6, and TNF-α) in LPS-activated RAW264.7 cells were evaluated. In addition, inhibitory activity of this class of compound on the chemokine MDC in IFN-γ-activated HaCaT cells, which is involved in chronic skin inflammation such a atopic dermatitis (AD),[18] was also examined.

2. Chemistry

Seven different types of acyl chains and nine different amine headgroups were employed to examine the effects of the fatty acid component and amine headgroup on the activity. Based on previous studies on the SAR of bioactive fatty acid amides, it was suggested that both fatty acid component and amine headgroup of the molecule are important for their bioactivity.[19–23] Therefore, each fatty acid component was incorporated with different amine headgroups.

In addition to endogenous fatty acid amides, including *N*-acyl ethanolamines, *N*-acyl amino acids (lipoamino acids) and *N*-acyl dopamines, non-natural amine headgroups, including isopropyl amine, *R*-(−)-2-aminopropanol, chloroethyl amine, (±)-3-amino-1,2-propanediol, vanillylamine and serotonine, were also used for derivatization. All fatty acid amides were prepared using the same procedure. Briefly, the fatty acid amides were prepared by treating each fatty acid component with different amines in the presence of 2-(1*H*-benzotriazole-1-yl)-1,2,3,3-tetramethyluronium tetrafluoroborate (TBTU) and triethylamine (TEA) in ethyl acetate at room temperature.[24]

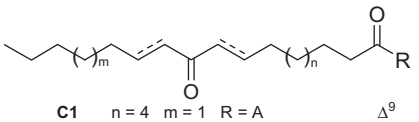
3. Results and discussion

The anti-inflammatory activity of endogenous fatty acid amides and their analogues (**1**–**78**) was evaluated by screening their inhibitory effects on NO production in LPS-activated RAW264.7 cells at preliminary concentrations of 50 and 100 μM. The data revealed only free enone fatty acids (**C1**–**C4**) and polyunsaturated fatty acids (**C7, C8**, and **C10**) to be active, and the fatty acid amides derived from these fatty acids generally exhibited significant activity. In addition, the amides of enone fatty acids (**1**–**30**) were more active than other analogues (Supplementary data). Regardless of the amine headgroup, the activity of three different acyl groups, group 1 (**1**–**30**) derived from enone fatty acids, group 2 (**31**–**46** and **64**–**70**) derived from saturated or monounsaturated fatty acids, and group 3 (**47**–**63** and **71**–**78**) derived from polyunsaturated fatty acids, was in the order of group 1 > group 3 > group 2, indicating that the bioactivity of fatty acid amides is strongly dependent on the nature of the fatty acid part of the molecule. However, all fatty acid amides with dopamine headgroup, *N*-acyl dopamines (**24**, **37**, **45**, **53**, **62**, **69**, and **77**), potently inhibited NO production, suggesting that a change in the amine headgroup can remarkably alter bioactivity profile of this class of compound, possibly by modulating cell permeability. The endogenous fatty acid amides and their analogues were also evaluated for their inhibitory activity on the chemokine MDC in IFN-γ-activated HaCaT cells. The *N*-acyl amino acids and *N*-acyl dopamines derived from enone fatty acids and ω-3 fatty acids were the most active with lower cytotoxicity to HaCaT cells than to RAW264.7 cells (Supplementary data).

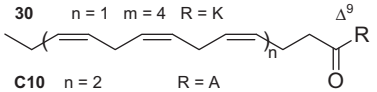
The amides of enone fatty acids (**1**–**30**) and *N*-acyl dopamines (**24**, **37**, **45**, **53**, **62**, **69**, and **77**) exhibited the highest inhibitory activity on NO production among the compounds tested. However, they were highly cytotoxic to RAW264.7 cells at concentrations of 50 and 100 μM. Hence, the observed inhibitory activity on NO production by these compounds might be biased by their cytotoxicity. Therefore, to clearly elaborate their anti-inflammatory activity, representative analogues of enone fatty acid amides (**2**, **6**, **10**, **14**, **18**, **21**, **22**, and **28**) and *N*-acyl dopamines (**24**, **37**, **45**, **53**, **62**, **69**, and **77**) were further examined for their ability to inhibit NO production at lower concentrations (3.125, 6.25, 12.5, and 25 μM). The amides of enone fatty acids (**2**, **6**, **10**, **14**, **18**, **21**, **22**, and **28**) significantly inhibited NO production in a concentration-dependent manner with *N*-acyl dopamines (**24**, **37**, **45**, **53**, **62**, **69**, and **77**) showing the most potent activity (Figure 1). A further evaluation of their inhibitory effects on pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6) showed that these compounds inhibit the production of IL-1β and IL-6 significantly in a concentration-dependent manner (Figures 3 and 4). However, suppression of

TNF-α production was not significant (Figure 2). According to the biological data, the activity of the enone fatty acid amides can be ranked in the order: **28** > **6**, **10**–**18**, **21**, **22** > **2**, **14**. This suggests that amine headgroups that can provide hydrogen bonding to biological targets may impart higher activity to the amides. A comparison of the activity of these compounds with previously known anti-inflammatory fatty acids amides revealed lipoamino acids **21** and **22** to be more active than the anti-inflammatory counterparts **52** and **76**, which were derived from ω-3 fatty acids.[25,26] The prostaglandin ratios, in which the J series predominates over the E series, promote the resolution of inflammatory conditions. Polyunsaturated acids conjugates such as **52** and **76** induced such favorable ratios of 15d-PGJ_2 and PGE in cell media from which they displayed anti-inflammatory activity.[25,26] However, polyunsaturated fatty acids are chemically unstable. Therefore, enone fatty acids might be a better template for the development of bioactive fatty acid amides than polyunsaturated fatty acids.

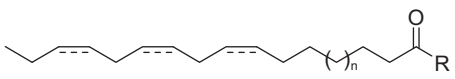
The *N*-acyl dopamines (**24**, **37**, **45**, **53**, **62**, **69**, and **77**) retained their potent inhibitory activity on NO production at non-cytotoxic concentrations (IC_{50} ~2 μM, TC_{50} ~13 μM). At similar concentration ranges, these *N*-acyl dopamines (**24**, **37**, **45**, **53**, **62**, **69**, and **77**) also potently suppressed the production of cytokines (TNF-α, IL-1β, and IL-6) in a concentration-dependent manner with more selectivity to IL-1β and IL-6 (Figures 5–7). Although structurally similar to *N*-acyl dopamines, *N*-acyl vanillylamines (**38**, **46**, **54**, **63**, **70**, and **78**), which contain a dopamine-like phenolic moiety, had no activity or much lower potency than *N*-acyl dopamines (Supplementary data). For example, compound **46** (olvanil), which is a structural analogue of capsaicin and possesses analgesic activity mediated by the vanilloid receptor type 1 (VR-1),[27] was much less active than compound **45**, suggesting that the dopamine moiety is needed to induce bioactivity. *N*-Acyl dopamines, such as *N*-palmitoyl (PALDA), *N*-stearoyl (STEARDA), *N*-oleoyl (OLDA) and *N*-arachidonoyl dopamines (NADA), have been characterized



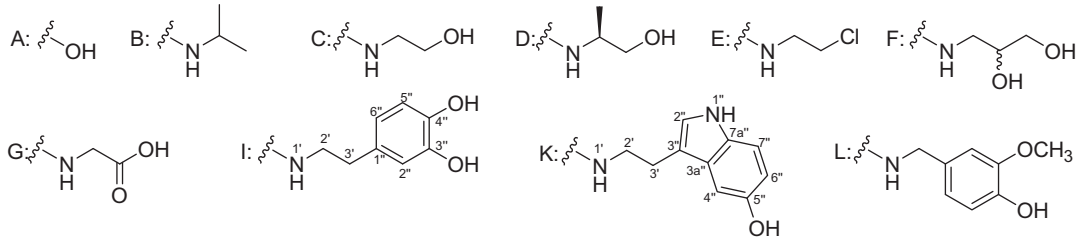
C1	n = 4	m = 1	R = A	Δ^9
C2	n = 3	m = 2	R = A	Δ^8
C3	n = 2	m = 3	R = A	Δ^{10}
C4	n = 1	m = 4	R = A	Δ^9
1	n = 4	m = 1	R = B	Δ^9
2	n = 3	m = 2	R = B	Δ^8
3	n = 2	m = 3	R = B	Δ^{10}
4	n = 1	m = 4	R = B	Δ^9
5	n = 4	m = 1	R = C	Δ^9
6	n = 3	m = 2	R = C	Δ^8
7	n = 2	m = 3	R = C	Δ^{10}
8	n = 1	m = 4	R = C	Δ^9
9	n = 4	m = 1	R = D	Δ^9
10	n = 3	m = 2	R = D	Δ^8
11	n = 2	m = 3	R = D	Δ^{10}
12	n = 1	m = 4	R = D	Δ^9
13	n = 4	m = 1	R = E	Δ^9
14	n = 3	m = 2	R = E	Δ^8
15	n = 2	m = 3	R = E	Δ^{10}
16	n = 1	m = 4	R = E	Δ^9
17	n = 4	m = 1	R = F	Δ^9
18	n = 3	m = 2	R = F	Δ^8
19	n = 2	m = 3	R = F	Δ^{10}
20	n = 1	m = 4	R = F	Δ^9
21	n = 2	m = 3	R = G	Δ^{10}
22	n = 1	m = 4	R = G	Δ^9
23	n = 4	m = 1	R = I	Δ^9
24	n = 3	m = 2	R = I	Δ^8
25	n = 2	m = 3	R = I	Δ^{10}
26	n = 1	m = 4	R = I	Δ^9
27	n = 4	m = 1	R = K	Δ^9
28	n = 3	m = 2	R = K	Δ^8
29	n = 2	m = 3	R = K	Δ^{10}
30	n = 1	m = 4	R = K	Δ^9



C10	n = 2	R = A
71	n = 2	R = B
72	n = 2	R = C
73	n = 2	R = D
74	n = 2	R = E
75	n = 2	R = F
76	n = 2	R = G
77	n = 2	R = I
78	n = 2	R = L



C5	n = 1	R = A	
C6	n = 3	R = A	Δ^9
C7	n = 3	R = A	$\Delta^{9,12}$
C8	n = 3	R = A	$\Delta^{9,12,15}$
C9	n = 7	R = A	
31	n = 1	R = B	
32	n = 1	R = C	
33	n = 1	R = D	
34	n = 1	R = E	
35	n = 1	R = F	
36	n = 1	R = G	
37	n = 1	R = I	
38	n = 1	R = L	
39	n = 3	R = B	Δ^9
40	n = 3	R = C	Δ^9
41	n = 3	R = D	Δ^9
42	n = 3	R = E	Δ^9
43	n = 3	R = F	Δ^9
44	n = 3	R = G	Δ^9
45	n = 3	R = I	Δ^9
46	n = 3	R = L	Δ^9
47	n = 3	R = B	$\Delta^{9,12,15}$
48	n = 3	R = C	$\Delta^{9,12,15}$
49	n = 3	R = D	$\Delta^{9,12,15}$
50	n = 3	R = E	$\Delta^{9,12,15}$
51	n = 3	R = F	$\Delta^{9,12,15}$
52	n = 3	R = G	$\Delta^{9,12,15}$
53	n = 3	R = I	$\Delta^{9,12,15}$
54	n = 3	R = L	$\Delta^{9,12,15}$
55	n = 3	R = K	$\Delta^{9,12,15}$
56	n = 3	R = B	$\Delta^{9,12}$
57	n = 3	R = C	$\Delta^{9,12}$
58	n = 3	R = D	$\Delta^{9,12}$
59	n = 3	R = E	$\Delta^{9,12}$
60	n = 3	R = F	$\Delta^{9,12}$
61	n = 3	R = G	$\Delta^{9,12}$
62	n = 3	R = I	$\Delta^{9,12}$
63	n = 3	R = L	$\Delta^{9,12}$
64	n = 7	R = B	Δ^{13}
65	n = 7	R = C	Δ^{13}
66	n = 7	R = D	Δ^{13}
67	n = 7	R = E	Δ^{13}
68	n = 7	R = F	Δ^{13}
69	n = 7	R = I	Δ^{13}
70	n = 7	R = L	Δ^{13}



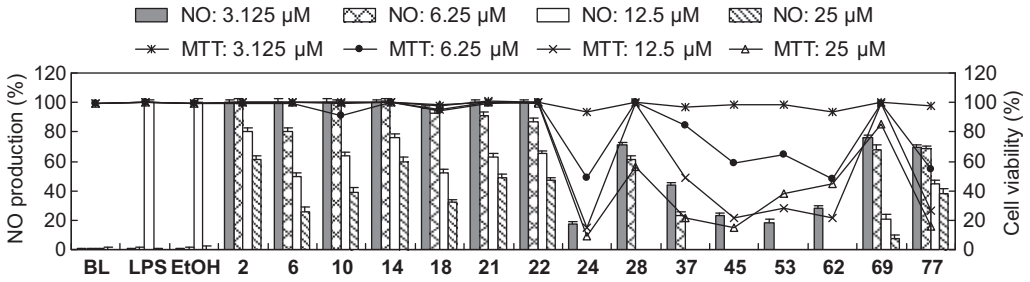


Figure 1. Inhibitory effects of selected fatty acid amides (**2**, **6**, **10**, **14**, **18**, **21**, **22**, **24**, **28**, **37**, **45**, **53**, **62**, **69**, and **77**) on the production of NO in LPS-activated RAW264.7 cells. The RAW264.7 cells (1.5×10^5 cells/mL) were stimulated with LPS (1 μg/mL) alone or with test samples at concentrations of 3.125, 6.25, 12.5, and 25 μM for 24 h (BL, blank). The nitric oxide production was determined using a Griess reagent. The cell viability was determined using the MTT method. The data represent the mean ± SD of triplicate experiments.

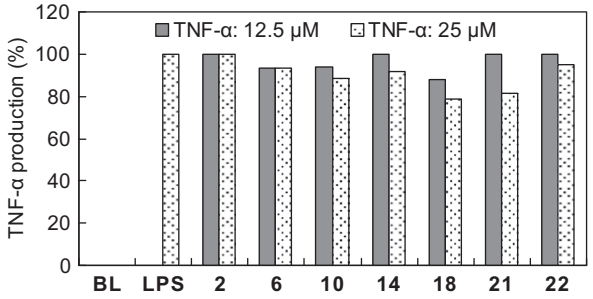


Figure 2. Inhibitory effects of selected fatty acid amides (**2**, **6**, **10**, **14**, **18**, **21**, and **22**) on the production of TNF-α in LPS-activated RAW264.7 cells. The RAW264.7 cells (1.5×10^5 cells/mL) were stimulated with LPS (1 μg/mL) alone or with test samples at concentrations of 12.5 and 25 μM for 24 h (BL, blank). The production of TNF-α was determined by ELISA. The data represent the mean ± SD of triplicate experiments.

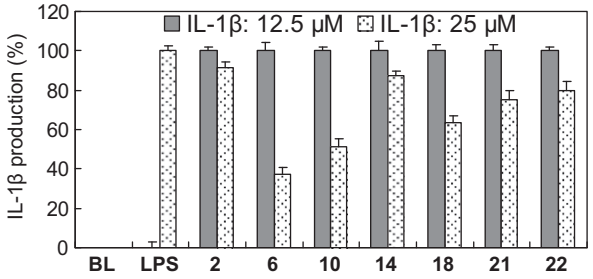


Figure 3. Inhibitory effects of selected fatty acid amides (**2**, **6**, **10**, **14**, **18**, **21**, and **22**) on the production of IL-1β in LPS-activated RAW264.7 cells. The RAW264.7 cells (1.5×10^5 cells/mL) were stimulated with LPS (1 μg/mL) alone or with test samples at concentrations of 12.5 and 25 μM for 24 h (BL, blank). The production of IL-1β was determined by ELISA. The data represent the mean ± SD of triplicate experiments.

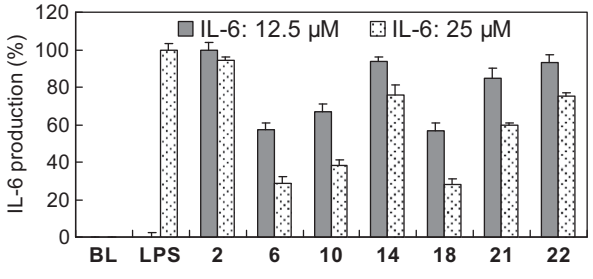


Figure 4. Inhibitory effects of selected fatty acid amides (**2**, **6**, **10**, **14**, **18**, **21**, and **22**) on the production of IL-6 in LPS-activated RAW264.7 cells. The RAW264.7 cells (1.5×10^5 cells/mL) were stimulated with LPS (1 μg/mL) alone or with test samples at concentrations of 12.5 and 25 μM for 24 h (BL, blank). The production of IL-6 was determined by ELISA. The data represent the mean ± SD of triplicate experiments.

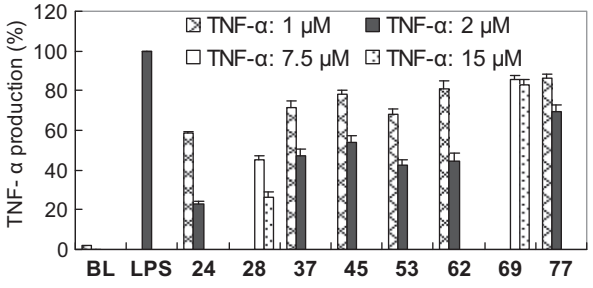


Figure 5. Inhibitory effects of *N*-acyl serotonine **28** and *N*-acyl dopamines (**24**, **37**, **45**, **53**, **62**, **69**, and **77**) on the production of TNF-α in LPS-activated RAW264.7 cells. The RAW264.7 cells (1.5×10^5 cells/mL) were stimulated with LPS (1 μg/mL) alone or with test samples at concentrations of 1.0 and 2.0 μM (**24**, **37**, **45**, **53**, **62**, and **77**) and 7.5 and 15 μM (**28** and **69**) for 24 h (BL, blank). The production of TNF-α was determined by ELISA. The data represent the mean ± SD of triplicate experiments.

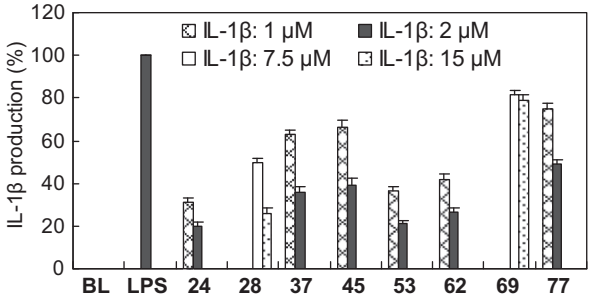


Figure 6. Inhibitory effects of *N*-acyl serotonine **28** and *N*-acyl dopamines (**24**, **37**, **45**, **53**, **62**, **69**, and **77**) on the production of IL-1β in LPS-activated RAW264.7 cells. The RAW264.7 cells (1.5×10^5 cells/mL) were stimulated with LPS (1 μg/mL) alone or with test samples at concentrations of 1.0 and 2.0 μM (**24**, **37**, **45**, **53**, **62**, and **77**) and 7.5 and 15 μM (**28** and **69**) for 24 h (BL, blank). The production of IL-1β was determined by ELISA. The data represent the mean ± SD of triplicate experiments.

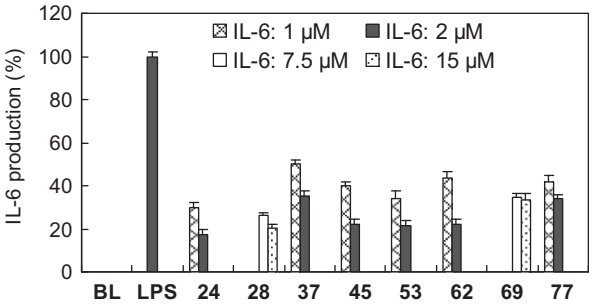


Figure 7. Inhibitory effects of *N*-acyl serotonine **28** and *N*-acyl dopamines (**24**, **37**, **45**, **53**, **62**, **69**, and **77**) on the production of IL-6 in LPS-activated RAW264.7 cells. The RAW264.7 cells (1.5×10^5 cells/mL) were stimulated with LPS (1 μg/mL) alone or with test samples at concentrations of 1.0 and 2.0 μM (**24**, **37**, **45**, **53**, **62**, and **77**) and 7.5 and 15 μM (**28** and **69**) for 24 h (BL, blank). The production of IL-6 was determined by ELISA. The data represent the mean ± SD of triplicate experiments.

from mammalian brain.[28] The implications of these compounds in inflammatory and neuropathic pain have been attributed to their strong effects on the transient receptor potential channel type 1 (TRPV-1). Two members of this family, OLDA and NADA, were shown to potently activate TRPV-1, also known as VR-1 for capsaicin. The other two congeners, PALDA and STEARDA, play a role as entourage compounds, which increase the activity of the proposed endovalilloids OLDA and NADA.[28] Quite recently, the anti-inflammatory activity of endogenous *N*-acyl dopamines in the central nervous system was reported.[29] NADA, in which the dopamine moiety is crucial for bioactivity, was found to activate a redox-sensitive p38 MAPK pathway that stabilizes COX-2 mRNA resulting in the accumulation of the COX-2 protein, and the activity is independent of CB-1 and TRPV-1 activation. In addition, NADA inhibited the expression of microsomal prostaglandin E synthase-1 (mPGES-1) and the release of PGE, and upregulated the expression of lipocalin-type prostaglandin D synthase (L-PGDS), thereby enhancing the release of PGD.[29] Besides, *N*-acyl dopamines were also found to inhibit cancer cell proliferation.[30,31] The combined results suggest that *N*-acyl dopamines are potential anti-inflammatory and antitumor leads.

Overall, the biological data showed that the inhibitory effects of endogenous fatty acid amides and their analogues on pro-inflammatory mediators are strongly dependent on the nature of the fatty acid part of the molecule. As expected, the amides derived from enone fatty acids showed significant anti-inflammatory activity. In addition, a change in the amine headgroup also altered the bioactivity profile remarkably. Regarding the amine part of the molecule, *N*-acyl dopamines (**24**, **37**, **45**, **53**, **62**, **69**, and **77**) exhibited potent inhibitory activity on the production of pro-inflammatory mediators (NO, TNF-α, IL-1β, and IL-6). Figure 8 illustrates the structure-activity relationship of endogenous fatty acid amides and their analogues.

4. Conclusion

This study examined the inhibitory effects of endogenous fatty acid amides and their analogues on the production of pro-inflammatory mediators (NO, TNF-α, IL-1β, and IL-6) in LPS-activated RAW264.7 cells. The inhibitory activity of this class of compound on the chemokine MDC in IFN-γ-activated HaCaT cells was also examined. These results showed that the fatty acid component of the amide molecule is important for their activity. The amides derived from enone fatty acids showed significant activity as expected and were more active than other amide counterparts. In addition, regarding the amine part of the molecule, *N*-acyl dopamines (**24**, **37**, **45**, **53**, **62**, **69**, and **77**) exhibited the most potent inhibitory activity on the production of NO and cytokines (TNF-α, IL-1β, and IL-6). On the other hand, an evaluation of the inhibitory activity of all fatty acid amides (**1**–**78**) on the production of MDC in IFN-γ-activated HaCaT cells revealed *N*-acyl dopamines and *N*-acyl amino acids derived from enone and ω-3 fatty acids to be the most active. Considering biological activity and chemical stability, this study suggests that the derivatization of endogenous fatty acid amides through the incorporation of enone fatty acids may provide potential anti-inflammatory leads.

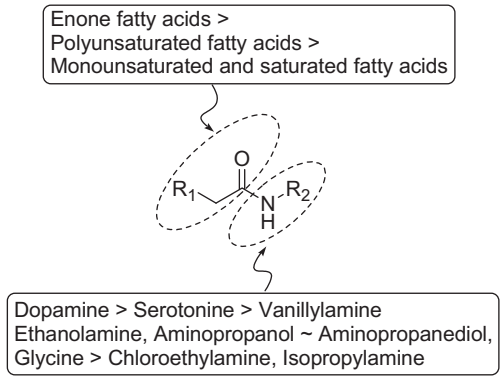


Figure 8. Structure–activity relationships of endogenous fatty acid amides and their analogues. The activity was determined from their ability to inhibit the production of pro-inflammatory mediators (NO, IL-6, IL-1β, and TNF-α).

5. Experimental

5.1. General

The ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on Varian Inova 400 spectrometer. The chemical shifts are reported with reference to the respective residual solvent or deuterated solvent peaks (δ_H 7.24 and δ_C 77.0 for $CDCl_3$; δ_H 3.30 and δ_C 49.0 for CD_3OD). The FABMS data were obtained on a JEOL JMS SX-102A spectrometer. HPLC was performed on 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. All chemical reagents were purchased from the Sigma-Aldrich and used as received.

5.2. Synthesis of fatty acid amides

5.2.1. (*E*)-*N*-Isopropyl-11-oxo-octadec-9-enamide (1)

All fatty acid amides with different acyl chains and amine heads were synthesized using the same procedure but with different precursor compounds. 2-(1*H*-Benzotriazole-1-yl)-1,2,3,3-tetramethyluronium tetrafluoroborate (TBTU, 1 equiv) was added to a mixture of compound **C1** (1 equiv) and triethylamine (TEA, 2 equiv) in EtOAc. 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 that was purified by reversed-phase HPLC (YMC ODS-H80) eluting with 80% aqueous CH_3CN to yield compound **1** (1.5 mg, 63%); colorless oil; ^{1}H NMR ($CDCl_3$, 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), 5.19 (1H, br s, NH), 4.07 (1H, m, –CH(CH_3)$_2$), 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), 1.34–1.25 (14H, m, CH_2), 1.13 (6H, d, J = 6.8 Hz, –CH(CH_3)$_2$), 0.89 (3H, t, J = 6.8 Hz, H-18); FABMS *m/z* 360 $[M+Na]^+$.

5.2.2. (*E*)-*N*-Isopropyl-10-oxo-octadec-8-enamide (2)

Colorless oil; ^{1}H NMR ($CDCl_3$, 400 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), 5.18 (1H, br s, NH), 4.08 (1H, m, –CH(CH_3)$_2$), 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), 1.36–1.28 (14H, m, CH_2), 1.13 (6H, d, J = 6.8 Hz, –CH(CH_3)$_2$), 0.92 (3H, t, J = 6.5 Hz, H-18); FABMS *m/z* 360 $[M+Na]^+$.

5.2.3. (*E*)-*N*-Isopropyl-9-oxo-octadec-10-enamide (3)

Colorless oil; ^{1}H NMR ($CDCl_3$, 400 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), 5.17 (1H, br s, NH), 4.06 (1H, m, –CH(CH_3)$_2$), 2.59 (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), 1.13 (6H, d, J = 6.8 Hz, –CH(CH_3)$_2$), 0.90 (3H, t, J = 7.0 Hz, H-18); FABMS *m/z* 360 $[M+Na]^+$.

5.2.4. (*E*)-*N*-Isopropyl-8-oxo-octadec-9-enamide (4)

Colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.93 (1H, dt, J = 16.0, 7.6 Hz, H-10), 6.11 (1H, dt, J = 16.0, 1.4 Hz, H-9), 5.20 (1H, br s, NH),

4.05 (1H, m, –*CH*(CH_3)$_2$), 2.57 (2H, t, *J* = 7.2 Hz, H-7), 2.27 (4H, m, H-2, H-11), 1.58 (4H, m, H-3, H-6), 1.46 (2H, m, H-12), 1.35–1.25 (14H, m, CH_2), 1.13 (6H, d, *J* = 6.8 Hz, –CH(*CH_3*)$_2$), 0.89 (3H, t, *J* = 6.8 Hz, H-18); FABMS *m/z* 360 $[M+Na]^+$.

5.2.5. (*E*)-*N*-Hydroxyethyl-11-oxo-octadec-9-enamide (5)

Colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.93 (1H, dt, *J* = 16.4, 7.2 Hz, H-9), 6.12 (1H, dt, *J* = 16.4, 1.2 Hz, H-10), 5.93 (1H, br s, NH), 3.73 (2H, t, *J* = 5.6 Hz, –*CH_2*CH_2OH), 3.44 (2H, quint, *J* = 6.0 Hz, –*CH_2*OH), 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), 1.37–1.26 (14H, m, CH_2), 0.89 (3H, t, *J* = 6.8 Hz, H-18); FABMS *m/z* 362 $[M+Na]^+$.

5.2.6. (*E*)-*N*-Hydroxyethyl-10-oxo-octadec-8-enamide (6)

Colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.91 (1H, dt, *J* = 16.0, 6.5 Hz, H-8), 6.12 (1H, dt, *J* = 16.0, 1.5 Hz, H-9), 5.92 (1H, br s, NH), 3.74 (2H, t, *J* = 5.6 Hz, –*CH_2*CH_2OH), 3.46 (2H, quint, *J* = 6.0 Hz, –*CH_2*OH), 2.59 (2H, t, *J* = 7.5 Hz, H-11), 2.28 (4H, m, H-2, H-7), 1.62 (4H, m, H-3, H-12), 1.51 (2H, m, H-6), 1.36–1.25 (14H, m, CH_2), 0.91 (3H, t, *J* = 6.5 Hz, H-18); FABMS *m/z* 362 $[M+Na]^+$.

5.2.7. (*E*)-*N*-Hydroxyethyl-9-oxo-octadec-10-enamide (7)

Colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.93 (1H, dt, *J* = 14.0, 7.0 Hz, H-11), 6.10 (1H, dt, *J* = 14.0, 1.5 Hz, H-10), 5.91 (1H, br s, NH), 3.71 (2H, t, *J* = 5.6 Hz, –*CH_2*CH_2OH), 3.42 (2H, quint, *J* = 6.0 Hz, –*CH_2*OH), 2.56 (2H, t, *J* = 7.5 Hz, H-8), 2.26 (4H, m, H-2, H-12), 1.58 (4H, m, H-3, H-7), 1.48 (2H, m, H-13), 1.37–1.28 (14H, m, CH_2), 0.92 (3H, t, *J* = 7.0 Hz, H-18); FABMS *m/z* 362 $[M+Na]^+$.

5.2.8. (*E*)-*N*-Hydroxyethyl-8-oxo-octadec-9-enamide (8)

Colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.92 (1H, dt, *J* = 16.0, 7.6 Hz, H-10), 6.11 (1H, dt, *J* = 16.0, 1.4 Hz, H-9), 5.93 (1H, br s, NH), 3.75 (2H, t, *J* = 5.6 Hz, –*CH_2*CH_2OH), 3.41 (2H, quint, *J* = 6.0 Hz, –*CH_2*OH), 2.60 (2H, t, *J* = 7.2 Hz, H-7), 2.28 (4H, m, H-2, H-11), 1.59 (4H, m, H-3, H-6), 1.46 (2H, m, H-12), 1.35–1.22 (14H, m, CH_2), 0.91 (3H, t, *J* = 6.8 Hz, H-18); FABMS *m/z* 362 $[M+Na]^+$.

5.2.9. (*E*)-*N*-[(1*R*)-2-Hydroxy-1-methylethyl]-11-oxo-octadec-9-enamide (9)

Colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.91 (1H, dt, *J* = 16.4, 7.2 Hz, H-9), 6.13 (1H, dt, *J* = 16.4, 1.2 Hz, H-10), 5.66 (1H, br s, NH), 4.07 (1H, m, –*CH*(CH_3)CH_2OH), 3.66 (1H, m, –CH(CH_3)*CH_b*OH), 3.53 (1H, m, –CH(CH_3)*CH_a*OH), 2.56 (2H, t, *J* = 7.6 Hz, H-12), 2.25 (4H, m, H-2, H-8), 1.58 (4H, m, H-3, H-13), 1.47 (2H, m, H-7), 1.36–1.27 (14H, m, CH_2), 1.15 (3H, d, *J* = 6.8 Hz, –CH(*CH_3*)CH_2OH), 0.89 (3H, t, *J* = 6.8 Hz, H-18); FABMS *m/z* 376 $[M+Na]^+$.

5.2.10. (*E*)-*N*-[(1*R*)-2-Hydroxy-1-methylethyl]-10-oxo-octadec-8-enamide (10)

Colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.92 (1H, dt, *J* = 16.0, 6.5 Hz, H-8), 6.12 (1H, dt, *J* = 16.0, 1.5 Hz, H-9), 5.63 (1H, br s, NH), 4.04 (1H, m, –*CH*(CH_3)CH_2OH), 3.67 (1H, m, –CH(CH_3)*CH_b*OH), 3.54 (1H, m, –CH(CH_3)*CH_a*OH), 2.57 (2H, t, *J* = 7.5 Hz, H-11), 2.28 (4H, m, H-2, H-7), 1.66 (4H, m, H-3, H-12), 1.52 (2H, m, H-6), 1.37–1.31 (14H, m, CH_2), 1.18 (3H, d, *J* = 6.8 Hz, –CH(*CH_3*)CH_2OH), 0.92 (3H, t, *J* = 6.5 Hz, H-18); FABMS *m/z* 376 $[M+Na]^+$.

5.2.11. (*E*)-*N*-[(1*R*)-2-Hydroxy-1-methylethyl]-9-oxo-octadec-10-enamide (11)

Colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.91 (1H, dt, *J* = 14.0, 7.0 Hz, H-11), 6.11 (1H, dt, *J* = 14.0, 1.5 Hz, H-10), 5.65 (1H, br s, NH), 4.06 (1H, m, –*CH*(CH_3)CH_2OH), 3.68 (1H, m, –CH(CH_3)*CH_b*OH), 3.55 (1H, m, –CH(CH_3)*CH_a*OH), 2.60 (2H, t, *J* = 7.5 Hz, H-8), 2.27 (4H, m, H-2, H-12), 1.59 (4H, m, H-3, H-7), 1.49 (2H, m, H-13), 1.35–1.28 (14H, m, CH_2), 1.15 (3H, d, *J* = 6.8 Hz, –CH(*CH_3*)CH_2OH), 0.90 (3H, t, *J* = 7.0 Hz, H-18); FABMS *m/z* 376 $[M+Na]^+$.

5.2.12. (*E*)-*N*-[(1*R*)-2-Hydroxy-1-methylethyl]-8-oxo-octadec-9-enamide (12)

Colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.93 (1H, dt, *J* = 16.0, 7.6 Hz, H-10), 6.12 (1H, dt, *J* = 16.0, 1.4 Hz, H-9), 5.61 (1H, br s, NH), 4.08 (1H, m, –*CH*(CH_3)CH_2OH), 3.66 (1H, m, –CH(CH_3)*CH_b*OH), 3.55 (1H, m, –CH(CH_3)*CH_a*OH), 2.59 (2H, t, *J* = 7.2 Hz, H-7), 2.25 (4H, m, H-2, H-11), 1.57 (4H, m, H-3, H-6), 1.47 (2H, m, H-12), 1.37–1.21 (14H, m, CH_2), 1.16 (3H, d, *J* = 6.8 Hz, –CH(*CH_3*)CH_2OH), 0.91 (3H, t, *J* = 6.8 Hz, H-18); FABMS *m/z* 376 $[M+Na]^+$.

5.2.13. (*E*)-*N*-Chloroethyl-11-oxo-octadec-9-enamide (13)

Colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.92 (1H, dt, *J* = 16.4, 7.2 Hz, H-9), 6.13 (1H, dt, *J* = 16.4, 1.2 Hz, H-10), 5.83 (1H, br s, NH), 3.62 (4H, m, –(*CH_2*)$_2$Cl), 2.59 (2H, t, *J* = 7.6 Hz, H-12), 2.28 (4H, m, H-2, H-8), 1.57 (4H, m, H-3, H-13), 1.49 (2H, m, H-7), 1.36–1.30 (14H, m, CH_2), 0.91 (3H, t, *J* = 6.8 Hz, H-18); FABMS *m/z* 380 $[M+Na]^+$.

5.2.14. (*E*)-*N*-Chloroethyl-10-oxo-octadec-8-enamide (14)

Colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.93 (1H, dt, *J* = 16.0, 6.5 Hz, H-8), 6.13 (1H, dt, *J* = 16.0, 1.5 Hz, H-9), 5.81 (1H, br s, NH), 3.63 (4H, m, –(*CH_2*)$_2$Cl), 2.56 (2H, t, *J* = 7.5 Hz, H-11), 2.29 (4H, m, H-2, H-7), 1.64 (4H, m, H-3, H-12), 1.53 (2H, m, H-6), 1.37–1.33 (14H, m, CH_2), 0.93 (3H, t, *J* = 6.5 Hz, H-18); FABMS *m/z* 380 $[M+Na]^+$.

5.2.15. (*E*)-*N*-Chloroethyl-9-oxo-octadec-10-enamide (15)

Colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.91 (1H, dt, *J* = 14.0, 7.0 Hz, H-11), 6.12 (1H, dt, *J* = 14.0, 1.5 Hz, H-10), 5.80 (1H, br s, NH), 3.65 (4H, m, –(*CH_2*)$_2$Cl), 2.61 (2H, t, *J* = 7.5 Hz, H-8), 2.28 (4H, m, H-2, H-12), 1.58 (4H, m, H-3, H-7), 1.48 (2H, m, H-13), 1.37–1.28 (14H, m, CH_2), 0.90 (3H, t, *J* = 7.0 Hz, H-18); FABMS *m/z* 380 $[M+Na]^+$.

5.2.16. (*E*)-*N*-Chloroethyl-8-oxo-octadec-9-enamide (16)

Colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.94 (1H, dt, *J* = 16.0, 7.6 Hz, H-10), 6.10 (1H, dt, *J* = 16.0, 1.4 Hz, H-9), 5.84 (1H, br s, NH), 3.62 (4H, m, –(*CH_2*)$_2$Cl), 2.58 (2H, t, *J* = 7.2 Hz, H-7), 1.59 (4H, m, H-3, H-6), 1.48 (2H, m, H-12), 1.35–1.24 (14H, m, CH_2), 0.89 (3H, t, *J* = 6.8 Hz, H-18); FABMS *m/z* 380 $[M+Na]^+$.

5.2.17. (*E*)-*N*-2,3-Dihydroxylpropyl-11-oxo-octadec-9-enamide (17)

Colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.91 (1H, dt, *J* = 16.4, 7.2 Hz, H-9), 6.13 (1H, dt, *J* = 16.4, 1.2 Hz, H-10), 5.98 (1H, br s, NH), 3.77 (1H, m, –CH_2*CH*(OH)CH_2OH), 3.53 (2H, m, –*CH_2*CH(OH)-CH_2OH), 3.44 (2H, m, –CH_2CH(OH)*CH_2*OH), 2.59 (2H, t, *J* = 7.6 Hz, H-12), 2.26 (4H, m, H-2, H-8), 1.59 (4H, m, H-3, H-13), 1.49 (2H, m, H-7), 1.35–1.30 (14H, m, CH_2), 0.89 (3H, t, *J* = 6.8 Hz, H-18); FABMS *m/z* 392 $[M+Na]^+$.

5.2.18. (*E*)-*N*-2,3-Dihydroxylpropyl-10-oxo-octadec-8-enamide (18)

Colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.92 (1H, dt, *J* = 16.0, 6.5 Hz, H-8), 6.13 (1H, dt, *J* = 16.0, 1.5 Hz, H-9), 5.97 (1H, br s, NH), 3.75 (1H, m, –CH_2*CH*(OH)CH_2OH), 3.55 (2H, m, –*CH_2*CH(OH)-CH_2OH), 3.42 (2H, m, –CH_2CH(OH)*CH_2*OH), 2.59 (2H, t, *J* = 7.5 Hz, H-11), 2.25 (4H, m, H-2, H-7), 1.62 (4H, m, H-3, H-12), 1.51 (2H, m, H-6), 1.36–1.27 (14H, m, CH_2), 0.91 (3H, t, *J* = 6.5 Hz, H-18); FABMS *m/z* 392 $[M+Na]^+$.

5.2.19. (*E*)-*N*-2,3-Dihydroxylpropyl-9-oxo-octadec-10-enamide (19)

Colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.94 (1H, dt, J = 14.0, 7.0 Hz, H-11), 6.10 (1H, dt, J = 14.0, 1.5 Hz, H-10), 5.95 (1H, br s, NH), 3.72 (1H, m, –CH_2*CH*(OH)CH_2OH), 3.53 (2H, m, –*CH_2*CH(OH)-CH_2OH), 3.42 (2H, m, –CH_2CH(OH)*CH_2*OH), 2.58 (2H, t, J = 7.5 Hz, H-8), 2.28 (4H, m, H-2, H-12), 1.59 (4H, m, H-3, H-7), 1.49 (2H, m, H-13), 1.35–1.28 (14H, m, CH_2), 0.90 (3H, t, J = 7.0 Hz, H-18); FABMS *m/z* 392 $[M+Na]^+$.

5.2.20. (*E*)-*N*-2,3-Dihydroxylpropyl-8-oxo-octadec-9-enamide (20)

Colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.93 (1H, dt, J = 16.0, 7.6 Hz, H-10), 6.12 (1H, dt, J = 16.0, 1.4 Hz, H-9), 5.94 (1H, br s, NH), 3.76 (1H, m, –CH_2*CH*(OH)CH_2OH), 3.51 (2H, m, –*CH_2*CH(OH)-CH_2OH), 3.40 (2H, m, –CH_2CH(OH)*CH_2*OH), 2.58 (2H, t, J = 7.2 Hz, H-7), 2.29 (4H, m, H-2, H-11), 1.59 (4H, m, H-3, H-6), 1.48 (2H, m, H-12), 1.37–1.21 (14H, m, CH_2), 0.89 (3H, t, J = 6.8 Hz, H-18); FABMS *m/z* 392 $[M+Na]^+$.

5.2.21. (*E*)-*N*-Ethanoyl-9-oxo-octadec-10-enamide (21)

White amorphous; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.91 (1H, dt, J = 14.0, 7.0 Hz, H-11), 6.27 (1H, br s, NH), 6.13 (1H, dt, J = 14.0, 1.5 Hz, H-10), 4.07 (2H, d, J = 4.8 Hz –*CH_2*COOH), 2.56 (2H, t, J = 7.5 Hz, H-8), 2.27 (4H, m, H-2, H-12), 1.59 (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* 376 $[M+Na]^+$.

5.2.22. (*E*)-*N*-Ethanoyl-8-oxo-octadec-9-enamide (22)

White amorphous; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.94 (1H, dt, J = 16.0, 7.6 Hz, H-10), 6.26 (1H, br s, NH), 6.10 (1H, dt, J = 16.0, 1.4 Hz, H-9), 4.05 (2H, d, J = 4.8 Hz –*CH_2*COOH), 2.59 (2H, t, J = 7.2 Hz, H-7), 2.25 (4H, m, H-2, H-11), 1.57 (4H, m, H-3, H-6), 1.48 (2H, m, H-12), 1.36–1.21 (14H, m, CH_2), 0.91 (3H, t, J = 6.8 Hz, H-18); FABMS *m/z* 376 $[M+Na]^+$.

5.2.23. (*E*)-*N*-[(3,4-Dihydroxyphenyl)ethyl]-11-oxo-octadec-9-enamide (23)

Colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.92–6.82 (2H, m, H-9 and H-5″), 6.73 (1H, s, H-2″), 6.59 (1H, d, J = 7.6 Hz, H-6″), 6.13 (1H, dt, J = 16.0, 1.4 Hz, H-10), 5.56 (1H, br s, NH), 3.49 (2H, q, J = 6.4 Hz, H-2′), 2.67 (2H, t, J = 6.4 Hz, H-3′), 2.58 (2H, t, J = 7.6 Hz, H-12), 2.27 (4H, m, H-2, H-8), 1.58 (4H, m, H-3, H-13), 1.47 (2H, m, H-7), 1.36–1.30 (14H, m, CH_2), 0.90 (3H, t, J = 6.8 Hz, H-18); FABMS *m/z* 454 $[M+Na]^+$.

5.2.24. (*E*)-*N*-[(3,4-Dihydroxyphenyl)ethyl]-10-oxo-octadec-8-enamide (24)

Colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.93–6.83 (2H, m, H-8 and H-5″), 6.72 (1H, s, H-2″), 6.58 (1H, d, J = 7.6 Hz, H-6″), 6.11 (1H, dt, J = 16.0, 1.4 Hz, H-9), 5.56 (1H, br s, NH), 3.47 (2H, q, J = 6.4 Hz, H-2′), 2.66 (2H, t, J = 6.4 Hz, H-3′), 2.60 (2H, t, J = 7.5 Hz, H-11), 2.27 (4H, m, H-2, H-7), 1.64 (4H, m, H-3, H-12), 1.52 (2H, m, H-6), 1.35–1.26 (14H, m, CH_2), 0.91 (3H, t, J = 6.5 Hz, H-18); FABMS *m/z* 454 $[M+Na]^+$.

5.2.25. (*E*)-*N*-[(3,4-Dihydroxyphenyl)ethyl]-9-oxo-octadec-10-enamide (25)

Colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.91–6.81 (2H, m, H-11 and H-5″), 6.71 (1H, s, H-2″), 6.59 (1H, d, J = 7.6 Hz, H-6″), 6.12 (1H, dt, J = 16.0, 1.4 Hz, H-10), 5.54 (1H, br s, NH), 3.45 (2H, q, J = 6.4 Hz, H-2′), 2.69 (2H, t, J = 6.4 Hz, H-3′), 2.58 (2H, t, J = 7.5 Hz, H-8), 2.29 (4H, m, H-2, H-12), 1.57 (4H, m, H-3, H-7), 1.47 (2H, m, H-13), 1.36–1.28 (14H, m, CH_2), 0.91 (3H, t, J = 7.0 Hz, H-18); FABMS *m/z* 454 $[M+Na]^+$.

5.2.26. (*E*)-*N*-[(3,4-Dihydroxyphenyl)ethyl]-8-oxo-octadec-9-enamide (26)

Colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.93–6.83 (2H, m, H-10 and H-5″), 6.72 (1H, s, H-2″), 6.57 (1H, d, J = 7.6 Hz, H-6″), 6.13 (1H, dt, J = 16.0, 1.4 Hz, H-9), 5.56 (1H, br s, NH), 3.46 (2H, q, J = 6.4 Hz, H-2′), 2.68 (2H, t, J = 6.4 Hz, H-3′), 2.58 (2H, t, J = 7.2 Hz, H-7), 2.26 (4H, m, H-2, H-11), 1.56 (4H, m, H-3, H-6), 1.49 (2H, m, H-12), 1.37–1.23 (14H, m, CH_2), 0.92 (3H, t, J = 6.8 Hz, H-18); FABMS *m/z* 454 $[M+Na]^+$.

5.2.27. (*E*)-*N*-[2-(5-Hydroxy-1*H*-indol-3-yl)ethyl]-11-oxo-octadec-9-enamide (27)

Colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.09 (1H, br s, NH), 7.21 (1H, d, J = 8.8 Hz, H-7″) 7.01–6.98 (2H, m, H-4″ and H-6″), 6.82–6.76 (2H, m, H-2″ and H-9), 6.12 (1H, dt, J = 16.0, 1.4 Hz, H-10), 5.59 (1H, br s, NH), 3.58 (2H, q, J = 6.8 Hz, H-2′), 2.88 (2H, t, J = 6.8 Hz, H-3′), 2.57 (2H, t, J = 7.6 Hz, H-12), 2.29 (4H, m, H-2, H-8), 1.60 (4H, m, H-3, H-13), 1.49 (2H, m, H-7), 1.35–1.30 (14H, m, CH_2), 0.91 (3H, t, J = 6.8 Hz, H-18); FABMS *m/z* 477 $[M+Na]^+$.

5.2.28. (*E*)-*N*-[2-(5-Hydroxy-1*H*-indol-3-yl)ethyl]-10-oxo-octadec-8-enamide (28)

Colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.03 (1H, br s, NH), 7.23 (1H, d, J = 8.8 Hz, H-7″) 7.01–6.94 (2H, m, H-4″ and H-6″), 6.87–6.76 (2H, m, H-2″ and H-8), 6.13 (1H, dt, J = 16.0, 1.4 Hz, H-9), 5.56 (1H, br s, NH), 3.58 (2H, q, J = 6.8 Hz, H-2′), 2.89 (2H, t, J = 6.8 Hz, H-3′), 2.57 (2H, t, J = 7.5 Hz, H-11), 2.28 (4H, m, H-2, H-7), 1.65 (4H, m, H-3, H-12), 1.54 (2H, m, H-6), 1.38–1.31 (14H, m, CH_2), 0.90 (3H, t, J = 6.5 Hz, H-18); FABMS *m/z* 477 $[M+Na]^+$.

5.2.29. (*E*)-*N*-[2-(5-Hydroxy-1*H*-indol-3-yl)ethyl]-9-oxo-octadec-10-enamide (29)

Colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.02 (1H, br s, NH), 7.23 (1H, d, J = 8.8 Hz, H-7″) 7.05–6.97 (2H, m, H-4″ and H-6″), 6.82–6.76 (2H, m, H-2″ and H-11), 6.11 (1H, dt, J = 16.0, 1.4 Hz, H-10), 5.56 (1H, br s, NH), 3.53 (2H, q, J = 6.8 Hz, H-2′), 2.90 (2H, t, J = 6.8 Hz, H-3′), 2.58 (2H, t, J = 7.5 Hz, H-8), 2.26 (4H, m, H-2, H-12), 1.59 (4H, m, H-3, H-7), 1.45 (2H, m, H-13), 1.36–1.26 (14H, m, CH_2), 0.89 (3H, t, J = 7.0 Hz, H-18); FABMS *m/z* 477 $[M+Na]^+$.

5.2.30. (*E*)-*N*-[2-(5-Hydroxy-1*H*-indol-3-yl)ethyl]-8-oxo-octadec-9-enamide (30)

Colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.07 (1H, br s, NH), 7.23 (1H, d, J = 8.8 Hz, H-7″) 7.06–6.90 (2H, m, H-4″ and H-6″), 6.87–6.76 (2H, m, H-2″ and H-10), 6.10 (1H, dt, J = 16.0, 1.4 Hz, H-9), 5.57 (1H, br s, NH), 3.58 (2H, q, J = 6.8 Hz, H-2′), 2.86 (2H, t, J = 6.8 Hz, H-3′), 2.57 (2H, t, J = 7.2 Hz, H-7), 2.27 (4H, m, H-2, H-11), 1.58 (4H, m, H-3, H-6), 1.46 (2H, m, H-12), 1.38–1.23 (14H, m, CH_2), 0.91 (3H, t, J = 6.8 Hz, H-18); FABMS *m/z* 477 $[M+Na]^+$.

5.3. Cell culture

The RAW 264.7 murine macrophage and HaCaT human keratinocytes were purchased from ATCC (Rockville, MD) and cultured in Dulbecco's modified Eagle's medium (DMEM) and Rosewell Park Memorial Institute (RPMI) medium, respectively, supplemented with 10% (v/v) heat-activated fetal bovine serum, streptomycin (100 μg/mL) and penicillin (100 U/mL) at 37 °C in a 5% CO_2 incubator.

5.4. Cytotoxicity assay

The 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. After 4 h, the cultures were removed from the incubator, and the formazan

crystals were dissolved by adding DMSO. The metabolic activity was quantified by measuring light absorbance at 540 nm.

5.5. Nitrite assay

The production of nitric oxide (NO) was measured, as described previously by Ryu et al.,[32] 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 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). The absorbance at 540 nm was measured using ELISA reader (Amersham Pharmacia Biotech, UK, USA) and the results were compared to a calibration curve using sodium nitrite as the standard.

5.6. Measurement of cytokines (IL-1β, IL-6, and TNF-α)

The inhibitory effects of fatty acid amides on IL-6, IL-1β, and TNF-α production were determined using the method described elsewhere.[33] The samples were dissolved in EtOH diluted with Dulbecco's modified essential medium (DMEM). The final concentration of chemical solvents did not exceed 0.1% in the culture medium. Under 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. LPS and the test materials were then added to the cultured cells. The medium was used for IL-6, IL-1β, and TNF-α assay using mouse ELISA kit (R & D Systems Inc., MN, USA).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.12.046.

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