Journal of Medicinal Chemistry

Article

Cite This: J. Med. Chem. 2019, 62, 9576−9592

pubs.acs.org/jmc

# Structure−Activity Relationships of Fish Oil Derivatives with Antiallergic Activity in Vitro and in Vivo

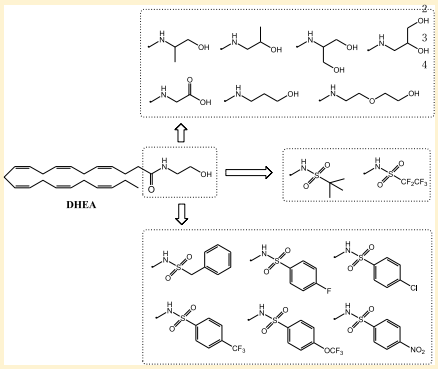
In-Hae Kim,[†] Yoshiki Kanayama,[†] Hisashi Nishiwaki,[†] Takuya Sugahara,[†,‡] and Kosuke Nishi*[,†,‡,§]

[†]Department of Bioscience, Graduate School of Agriculture and [‡]Food and Health Sciences Research Center, Ehime University, Matsuyama, Ehime 790-8566, Japan

[§]Research Unit for Skeletal Health and Diseases, Ehime University, Toon, Ehime 791-0295, Japan

Supporting Information

**ABSTRACT:** A series of unsaturated fatty acids in fish oil and their corresponding ethanolamide metabolites were explored to find active fish oil components of antiallergic activity in vitro. Ethanolamides of omega-3 fatty acids ($\alpha$-linolenic acid, EPA, and DHA) were found to possess promising antiallergic activity, whereas free fatty acids and ethanolamides of other fatty acids exhibited no or weak potency. Based on this finding, structure−activity relationships of DHA-ethanolamide (DHEA) derivatives were investigated to yield better fatty acid derivatives with enhanced antiallergic activity in vitro and in vivo. When the ethanolamide moiety of DHEA was replaced by the substituted sulfonamide functionality, highly promising potency was provided in vitro. Compound **59** showed improved antiallergic activity in vivo over DHEA. The results indicate that optimized DHEA derivatives have enhanced antiallergic activity in vitro and in vivo, and the resulting structures will be an important basis for further development of bioavailable derivatives with promising allergy suppressive activity.




Downloaded via UNIV OF LIVERPOOL on February 9, 2020 at 05:58:05 (UTC).
See https://pubs.acs.org/sharingguidelines for options on how to legitimately share published articles.

## INTRODUCTION

Allergy is one of the medical conditions triggered by stimulation of a wide range of allergens such as food, chemicals, and pollen. Mast cells of the immune system store a number of different chemical mediators including histamine and various proteins in coarse granules found throughout the cytoplasm of the cell. On the stimulation by an allergen, the mast cells release the contents of their granules by a process called degranulation.[1−3] The degranulated histamine induces a variety of allergic responses such as atopic, asthma, dermatitis, allergic rhinitis, and conjunctivitis.[4,5] For the last ten years, the number of people suffering from these allergic disorders has been increasing in the developed countries including Japan, underlying that proper protection or treatment of allergy is highly required.[6,7]

Fish oil is rich in omega-3 polyunsaturated fatty acids (PUFAs) such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). Studies indicate that fish oil and omega-3 PUFAs possess broad beneficial effects on human health. In vitro and in vivo anti-inflammatory effects of fish oil and omega-3 PUFAs are well addressed by some research groups.[8,9] The risk of cancers, cardiovascular disease, hypertension, and neurological disorders is known to be reduced by intake of fish oil or omega-3 PUFAs.[10−16] Recently, some studies have revealed that consumed DHA and EPA are endogenously converted to an ethanolamide metabolite, docosahexaenoyl ethanolamide (DHEA), and eicosapentaenoyl ethanolamide (EPEA), respectively, and the amide metabolites lead to the biological functions as a lipid mediator in vitro and in vivo.[11−13,16,17] This suggests that the endogenous ethanolamide metabolites of DHA and/or EPA are important mediators playing a role in showing their wide beneficial effects in the body and that they have a great potential to be used as pharmaceutical lead structures for the treatment of the omega-3 PUFA-related diseases. On the other hand, it has been also well known that fish oil or omega-3 PUFA intake is associated with allergy protection.[18−20] However, little is clear about the mode of the antiallergic effect of fish oil or omega-3 PUFAs. Based on reports, it can be assumed that ethanolamide metabolites of omega-3 PUFAs or other fatty acids in fish oil may play an important role in the control of allergy such as other omega-3 PUFA-related disorders, as well.

In the present study, a series of long-chain unsaturated fatty acids in fish oil and their corresponding ethanolamide metabolites were investigated to elucidate active components of fish oil with antiallergic activity in vitro. Based on the finding, extensive structure−activity relationship (SAR) studies were performed to discover further improved unsaturated fatty acid derivatives with enhanced antiallergic activity in vitro and in vivo.

## RESULTS AND DISCUSSION

**Chemistry.** Ethanolamides of unsaturated fatty acid components of fish oil (**12**, **13**, and **17** in Table 1) were synthesized as seen in Scheme 1. 2-Aminoethanol was coupled with a corresponding fatty acid by using 1-[3-(dimethylamino)-propyl]-3-ethylcarbodiimide hydrochloride (EDCI) in the

Received: June 23, 2019
Published: October 16, 2019

 © 2019 American Chemical Society 

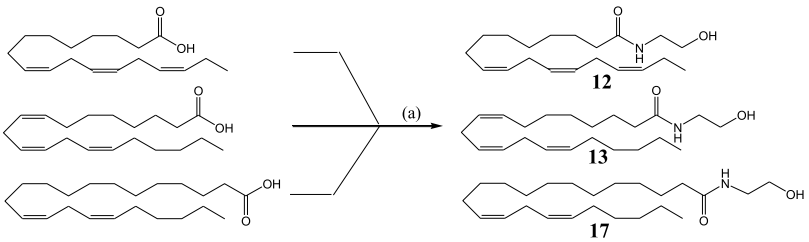
DOI: 10.1021/acs.jmedchem.9b00994
J. Med. Chem. 2019, 62, 9576−9592

**Table 1. Anti-degranulating Activity of Unsaturated Fatty Acids in Fish Oil and Their Corresponding Ethanolamides in Mast Cells**

| No. | Fatty acid | Structure | $IC_{50}$ (μM) [a] | No. | Structure | $IC_{50}$ (μM) [a] |
|---|---|---|---|---|---|---|
| **1** | Oleic acid (C18:1) | | >50 | **10** | | >50 |
| **2** | Linoleic acid (C18:2) | | >50 | **11** | | 48 |
| **3** | α-Linolenic acid (C18:3) | | >50 | **12** | | 29 |
| **4** | *cis*-8,11,14-Eicosatrienoic acid (C20:3) | | >50 | **13** | | >50 |
| **5** | Arachidonic acid (C20:4) | | >50 | **14** | | >50 |
| **6** | EPA (C20:5) | | >50 | **15** [b] | | 24 |
| **7** | 13*Z*-Docosaenoic acid (C22:1) | | >50 | **16** | | >50 |
| **8** | *cis*-13,16-Docosadienoic acid (C22:2) | | >50 | **17** | | >50 |
| **9** | DHA (C22:6) | | >50 | **18** [c] | | 25 |

[a]Anti-degranulating activity was evaluated by a *β*-hexosaminidase release assay. [b]Eicosapentaenoyl ethanolamide (EPEA). [c]Docosahexaenoyl ethanolamide (DHEA).

**Scheme 1. Syntheses of Ethanolamides of Unsaturated Fatty Acids**[a]



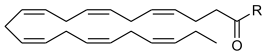

[a]Reagents and conditions: (a) 2-aminoethanol, EDCI, DMAP, dichloromethane, room temp.

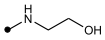
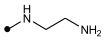
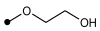
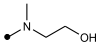
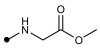
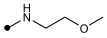
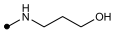
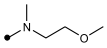
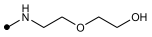
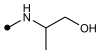
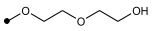
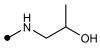
presence of 4-dimethylaminopyridine (DMAP) in dichloromethane to afford an ethanolamide of fatty acid in approximately 80% yield.[21] By using the same reaction as that described in Scheme 1, a number of amide or ester derivatives of DHA (DHEA, **20**−**23**, **26**−**30**, **34**−**36** in Table 2, **37**−**50** in Table 3, and **51**−**62** in Table 4) were prepared as depicted in Scheme 2. A corresponding amine or alcohol was added to a solution of DHA and EDCI in dichloromethane in the presence of DMAP as a base to provide an amide or ester derivative in a range of 70−95% yield.

Compound **19** was synthesized as outlined in Scheme 3. The coupling of DHA with *tert*-butyl 2-aminoethylcarbamate in the presence of EDCI and DMAP in dichloromethane gave a N-proptected amine−amide intermediate, which was treated with trifluoroacetic acid in dichloromethane to yield compound **19** in 78% yield.[22] Syntheses of mono- (**24**) or di-methyl (**25**)-substituted ethanolamide derivatives were carried out as outlined in Scheme 4. The reaction of 2-aminoethanol with di-*tert*-butyl dicarbonate in the presence of triethylamine as a base gave a N-protected ethanolamine intermediate, which was alkylated by using iodomethane in the presence of sodium


DOI: 10.1021/acs.jmedchem.9b00994
J. Med. Chem. 2019, 62, 9576−9592

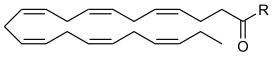
**Table 2. Anti-degranulating Activity of Alkylamide Derivatives of DHEA in Mast Cells**

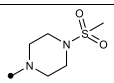
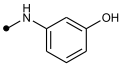
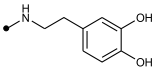
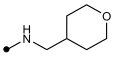
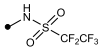


| No. | R | $IC_{50}$ (μM) [a] | No. | R | $IC_{50}$ (μM) [a] |
|---|---|---|---|---|---|
| DHEA |  | 25 | 28 | | 16 |
| 19 |  | >50 | 29 | | 17 |
| 20 | | >50 | 30 | | >50 |
| 21 | | >50 | 31 | | >50 |
| 22 |  | >50 | 32 | | 24 |
| 23 |  | >50 | 33 |  | >50 |
| 24 |  | >50 | 34 |  | 18 |
| 25 |  | >50 | 35 |  | 18 |
| 26 |  | 24 | 36 |  | >50 |
| 27 |  | 27 | | | |

[a]Anti-degranulating activity was evaluated by a β-hexosaminidase release assay.

**Table 3. Anti-degranulating Activity of Substituted Amide Derivatives of DHEA in Mast Cells**



| No. | R | $IC_{50}$ (μM) [a] | No. | R | $IC_{50}$ (μM) [a] |
|---|---|---|---|---|---|
| 37 | | >50 | 44 |  | >50 |
| 38 |  | >50 | 45 | | >50 |
| 39 |  | >50 | 46 | | 17 |
| 40 | | >50 | 47 | | >50 |
| 41 | | >50 | 48 | | >50 |
| 42 |  | >50 | 49 | | 47 |
| 43 | | >50 | 50 |  | 21 |

[a]Anti-degranulating activity was evaluated by β-hexosaminidase release assay.

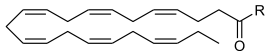
hydride in *N*,*N*-dimethylformamide (DMF) to provide a N-protected ether amine in 87% yield.[23] The protecting group of the ether amine was then removed by adding trifluoroacetic acid in dichloromethane to afford an ether amine, followed by coupling with DHA by using EDCI and DMAP in dichloromethane to make compound **24** in 82% yield.[22] Compound **25** was prepared in the same reactions as those used for the synthesis of compound **24** by using 2-(methylamino)ethanol as a starting material as shown in Scheme 4(II).

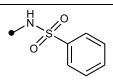
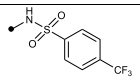
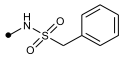
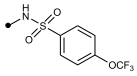
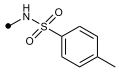
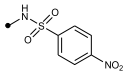
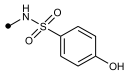
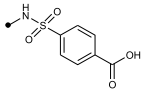
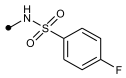
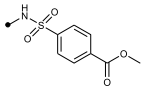
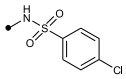
In order to yield a glycerol derivative (**31**), four reactions were conducted as depicted in Scheme 5. The reaction of glycerol with *tert*-butyldimethylchlorosilane in dichloromethane in the



DOI: 10.1021/acs.jmedchem.9b00994
J. Med. Chem. 2019, 62, 9576−9592

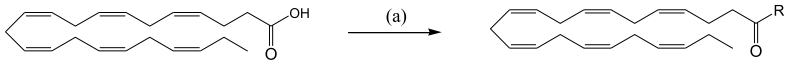
**Table 4. Anti-degranulating Activity of Arylamide Derivatives of DHEA in Mast Cells**



| No. | R | $IC_{50}$ (μM)[a] | No. | R | $IC_{50}$ (μM)[a] |
|---|---|---|---|---|---|
| **51** |  | >50 | **57** |  | 15 |
| **52** |  | 22 | **58** |  | 19 |
| **53** |  | 27 | **59** |  | 15 |
| **54** |  | >50 | **60** |  | >50 |
| **55** |  | 24 | **61** |  | 30 |
| **56** |  | 24 | **62** | | >50 |

[a]Anti-degranulating activity was evaluated by β-hexosaminidase release assay.

**Scheme 2. Syntheses of Amide or Ester Derivatives of DHA**[a]



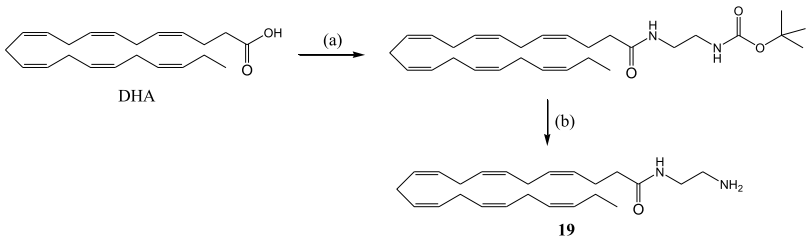
DHA

R: amine or alcohol

- **DHEA**, **20-23**, **26-30**, **34-36** in Table **2**
- **37-50** in Table **3**
- **51-62** in Table **4**

[a]Reagents and conditions: (a) a corresponding amine or ester, EDCI, DMAP, $CH_2Cl_2$, room temp.

**Scheme 3. Synthesis of (4Z,7Z,10Z,13Z,16Z,19Z)-*N*-(2-Aminoethyl)docosa-4,7,10,13,16,19-hexaenamide (19)**[a]



DHA

**19**

[a]Reagents and conditions: (a) *tert*-butyl 2-aminoethylcarbamate, EDCI, DMAP, $CH_2Cl_2$, room temp; (b) TFA, $CH_2Cl_2$, room temp.

presence of DMAP and triethylamine synthesized a protected glycerol intermediate. The free secondary alcohol of the intermediate was coupled with DHA by using EDCI and DMAP in dichloromethane to provide a protected glycerol ester of DHA, followed by the reaction of tetrabutylammonium fluoride in tetrahydrofuran (THF) to afford compound **31** in 35% overall yield.

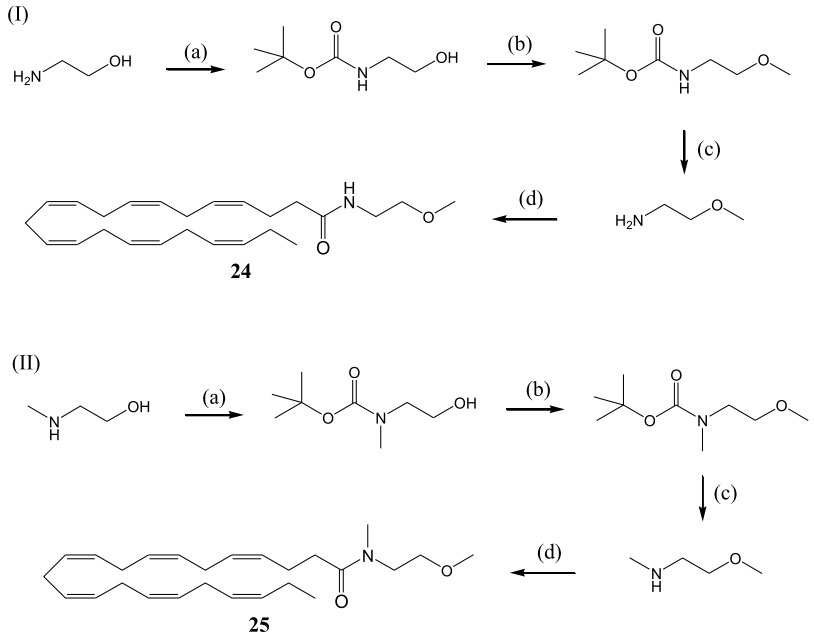
Syntheses of acetate (**33**) and acetic acid (**32**) derivatives were carried out as shown in Scheme 6. Glycine was reacted with chlorotrimethylsilane in methanol to prepare the corresponding methyl ester hydrochloride,[24] which was then used for the coupling with DHA by using EDCI and DMAP in the presence of triethylamine in dichloromethane to afford compound **33** in approximately 97% yield. Compound **33** was hydrolyzed by using 1 N sodium hydroxide in methanol to give the corresponding acid derivative (**32**) in 98% yield.

**Structure–Activity Relationships.** Although DHA and EPA are major components of fish oil, a wide range of saturated and unsaturated fatty acids exist in fish oil, and proportions of the fatty acids vary depending on fish species,[25,26] implying that the antiallergic effect of fish oil can emerge from not only DHA or EPA but also other lipids in fish oil. In this study, we especially focused on unsaturated fish oil components including DHA and EPA for the evaluation of antiallergic activity. Because, as



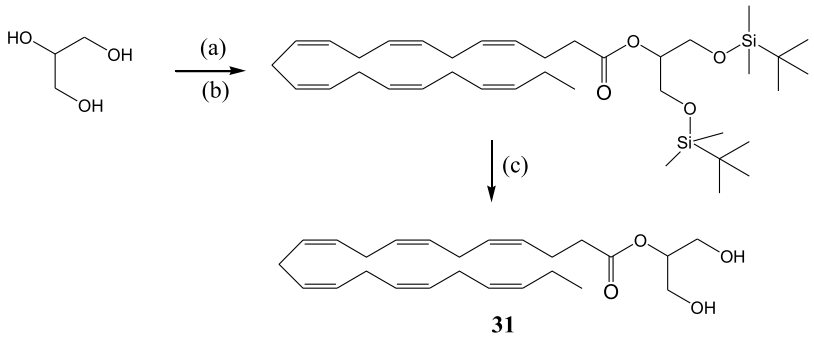
DOI: 10.1021/acs.jmedchem.9b00994
J. Med. Chem. 2019, 62, 9576−9592

**Scheme 4. Syntheses of (4Z,7Z,10Z,13Z,16Z,19Z)-*N*-(2-Methoxyethyl)docosa-4,7,10,13,16,19-hexaenamide (24) and (4Z,7Z,10Z,13Z,16Z,19Z)-*N*-(2-Methoxyethyl)-*N*-methyldocosa-4,7,10,13,16,19-hexaenamide (25)[a]**



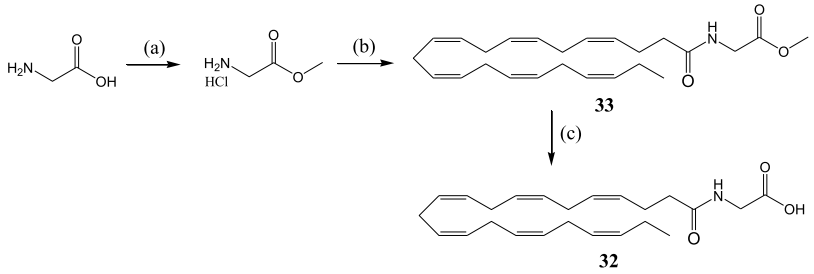

[a]Reagents and conditions: (a) $(Boc)_2O$, $Et_3N$, $CH_2Cl_2$, room temp; (b) NaH, $CH_3I$, DMF, room temp; (c) TFA, $CH_2Cl_2$, room temp; (d) $Et_3N$, 0 °C, then DHA, EDCI, DMAP, $CH_2Cl_2$, room temp.

**Scheme 5. Synthesis of (4Z,7Z,10Z,13Z,16Z,19Z)-1,3-Dihydroxypropan-2-yl Docosa-4,7,10,13,16,19-hexaenoate (31)[a]**




[a]Reagents and conditions: (a) TBSCl, DMAP, $Et_3N$, $CH_2Cl_2$, room temp; (b) DHA, EDCI, DMAP, $CH_2Cl_2$, room temp; (c) TBAF, THF, 0 °C.

**Scheme 6. Syntheses of Methyl 2-((4Z,7Z,10Z,13Z,16Z,19Z)-docosa-4,7,10,13,16,19-hexaenamido)acetate (33) and 2-((4Z,7Z,10Z,13Z,16Z,19Z)-Docosa-4,7,10,13,16,19-hexaenamido)acetic Acid (32)[a]**




[a]Reagents and conditions: (a) TMSCl, MeOH, room temp; (b) DHA, EDCI, DMAP, $Et_3N$, $CH_2Cl_2$, room temp; (c) 1 N NaOH, MeOH, room temp.

previously indicated, the conversion of the free acid function of DHA or EPA to the corresponding ethanolamide is essential for leading to a variety of biological functions in the body,[11−13,16,17] nine different common unsaturated fatty acids in fish oil and their corresponding ethanolamide metabolites were examined for suppressive activity on mast cell degranulation using rat basophilic leukemia RBL-2H3 cells, which are commonly used as a model of mast cells.[27,28] As shown in Table 1, all free fatty acids (**1**−**9**) tested did not show any anti-degranulating activity. The omega-3 fatty acids, EPA (**6**) and DHA (**9**), which are the


DOI: 10.1021/acs.jmedchem.9b00994
J. Med. Chem. 2019, 62, 9576−9592

main components of fish oil, induced no anti-degranulating activity. Other omega-9, -6, or -3 fatty acids (**1**−**5**, **7**, and **8**) such as oleic acid, α-linolenic acid, and arachidonic acid were also found to have no activity. These results are in line with a previous study reporting that DHA, EPA, and arachidonic acid do not reduce IgE-mediated degranulation of human mast cell line LAD2 cells.[29] However, surprisingly, the incorporation of the ethanolamide function to the place of the free acid of the fatty acids produced anti-degranulating activity. Comparing C18 fatty acids with ethanolamide (**10**−**12**), an omega-9 fatty acid, oleic acid ethanolamide with one double bond (**10**) had no anti-degranulating activity. Ethanolamide of linoleic acid with two double bonds (**11**), an omega-6 fatty acid, showed weak activity. However, in an omega-3 fatty acid, α-linolenic acid ethanolamide with three double bonds (**12**), a significant enhancement in anti-degranulating activity was observed. It seems that three consecutive double bonds from the omega-3 position of C18 fatty acid ethanolamides (**12**) are important for affording antiallergic activity. When this finding was compared to the results of C20 (**13**−**15**) and C22 (**16**−**18**) fatty acid ethanolamides, a similar trend was observed. As seen in compounds **13** and **14**, C20 fatty acid ethanolamides that have three or four consecutive double bonds from the omega-6 position exhibited no activity, whereas ethanolamide of EPA (**15**), a C20 fatty acid with five consecutive double bonds from the omega-3 position, was effective for suppressing the allergic reaction. This indicates that not the number of double bonds but the position of the consecutive double bonds in fatty acids is more important for yielding antiallergic activity. On the other hand, Cruz et al.[30] have reported that ethanolamide of arachidonic acid (**14**) inhibits IgE-mediated degranulation of murine bone marrow-derived mast cells, indicating that a different result can be observed depending on the cells used for evaluating mast cell degranulation. Likewise, a C22 omega-9 fatty acid ethanolamide with one double bond (**16**) or a C22 omega-6 fatty acid ethanolamide with two double bonds (**17**) did not produce antiallergic activity. However, in a C22 omega-3 fatty acid ethanolamide with six consecutive double bonds from the omega-3 position (**18**; DHEA), similar antiallergic activity to that of C18 omega-3 (**12**) and C20 omega-3 fatty acid (**15**) ethanolamides was gained. These results indicate that at least three consecutive double bonds from the omega-3 position of C18 (**12**), C20 (**15**), or C22 (**18**) fatty acid ethanolamides are necessary to induce antiallergic activity and that the carbon chain length of the omega-3 fatty acids is not a critical factor to generate antiallergic activity. The results in Table 1 indicate that polyunsaturated omega-3 free fatty acids such as α-linolenic acid, EPA, or DHA are not effective for suppressing degranulation, but their ethanolamide metabolites play an important role in the control of the allergic process. This suggests that the corresponding ethanolamides of the omega-3 fatty acids are active mediators leading to antiallergic effects of fish oil, which is consistent with previous reports that ethanolamides of DHA and EPA are modulators with biological functions such as anti-inflammation or anticancer effect.[11−13,16,17] Moreover, unexpectedly, α-linolenic acid, which is not a major omega-3 fatty acid present less than 1% in fish oil, was found to possess promising antiallergic activity, implying that the antiallergic effect can be further discovered from unexpected fatty acid structures in fish oil or by structural optimizations. Although it has been well known that fish oil rich in DHA and EPA is effective for controlling allergic disorders, no studies verified its mode of action in the body. The finding in Table 1 elucidates that antiallergic effects of fish oil are derived from the action of ethanolamides of the omega-3 fatty acids. The SARs in Table 1, which were explored based on the part of the common fatty acids in fish oil, are useful for discovering new fatty acids or novel chemical structures with better antiallergic activity.

Based on the above results, we further tried to yield better omega-3 fatty acid derivatives with improved antiallergic activity in vitro and in vivo by using extensive SAR studies. Among omega-3 fatty acids with antiallergic activity found in this study, EPEA and DHEA yielded better activity than ethanolamide of α-linolenic acid. In addition, some studies indicate that DHEA is an important lipid mediator related to a variety of biological functions such as anticancer and anti-inflammation in in vitro and in vivo experiments,[8−17] which implies that DHEA derivatives with improved biological activity have a big potential to be used for the treatment of a wide range of DHEA-related disorders including allergy. Thus, DHEA was used as a lead structure for the SARs in this study. Structural optimizations were made in the ethanolamide moiety of DHEA fixing the long-chain lipid part of DHEA to increase antiallergic activity (Figure 1).

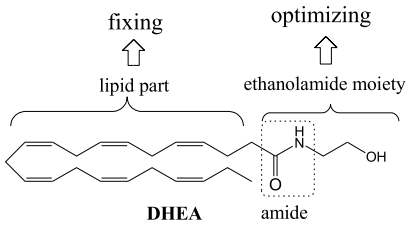



**Figure 1.** Structural optimization of (4Z,7Z,10Z,13Z,16Z,19Z)-*N*-(2-hydroxyethyl)docosa-4,7,10,13,16,19-hexaenamide (DHEA). The ethanolamide moiety of DHEA was optimized by a wide range of functional groups without modifying the unsaturated lipid part.

As shown in Table 2, a series of substituted or nonsubstituted alkylamide derivatives were first synthesized and their anti-degranulating activity was determined in the mast cells. As seen in compound **19**, anti-degranulating activity of DHEA disappeared when OH of the ethanolamide moiety was replaced by $NH_2$. No activity was also observed in compounds without OH (**20** and **21**), indicating that the OH group in the ethanolamide moiety of DHEA is important for retaining antiallergic activity. Replacing NH of the amide function in the ethanolamide moiety of DHEA by oxygen (**22**) dropped activity, indicating that amide functionality is necessary for the antiallergic action of DHEA. In order to see whether incorporating a substituent onto NH, OH, or the ethyl chain of the ethanolamide moiety affects antiallergic activity, a series of substituted derivatives were synthesized (**23**−**31**). When a methyl group was added to NH (**23**) or OH (**24**) of the ethanolamide moiety, no activity was induced. In compounds substituted with two methyl groups on both NH and OH (**25**), no significant activity was also observed, suggesting that substitution on the NH or OH group of the ethanolamide moiety is not favorable for improving potency and the hydrogen atoms on both the NH and OH groups play a highly important role in generating anti-degranulating activity. On the other hand, a methyl group incorporated onto the ethyl chain of the ethanolamide moiety did not affect potency (**26** and **27**). Similar (**26**) or a little lower (**27**) activity compared to that of DHEA was obtained, indicating that the ethyl chain in the ethanolamide


DOI: 10.1021/acs.jmedchem.9b00994
J. Med. Chem. 2019, 62, 9576−9592

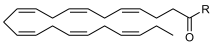
moiety can be an optimal position for substitutions to improve antiallergic activity. Interestingly, when a hydroxymethyl group (**28** and **29**) was introduced to the ethyl chain of the ethanolamide moiety instead of a methyl group (**26** and **27**), significantly enhanced activity over that of DHEA was gained. This suggests that a polar hydroxymethyl group is a highly useful substituent over a nonpolar methyl group for making better fatty acid derivatives with improved potency. Further, it was verified that the ethyl chain of the ethanolamide moiety is a suitable position for adding substituents to increase potency. However, a polar hydroxyethyl group present on NH of the amide function of the ethanolamide moiety (**30**) caused a big loss in activity, which was consistent with the result of compound **23** with a methyl group on NH of the amide. As expected, the corresponding ester analogue of compound **28** (**31**) possessed no activity, supporting again that amide function with no substituent is crucial for maintaining significant potency. As seen in compound **32**, acetic acid introduced instead of ethanol of DHEA led to similar antiallergic activity to that of DHEA, whereas the corresponding methyl acetate analogue (**33**) did not have any potency, which indicates that a polar functionality containing a hydrogen atom such as an acid or a primary alcohol is necessary for exhibiting decent antiallergic activity. Furthermore, increased potency over that of DHEA was exhibited when the ethanol of DHEA was replaced by propanol (**34**). A diethylene glycol derivative (**35**) had also similar potency to that of the propanol compound (**34**), suggesting that introducing alcohol functions with a longer alkyl chain than ethanol is very useful for enhancing antiallergic activity. Switching the amide function of compound **35** with potent activity to the corresponding ester (**36**) decreased activity a lot, which was similar to the results of the ester compounds **23** and **31**. The results in Table 2 show that antiallergic activity of DHEA is enhanced by optimizing the ethanolamide moiety by a series of substituted and nonsubstituted alkyl groups. It was found that two hydrogen atoms on both NH and OH of the ethanolamide moiety of DHEA are essential for maintaining antiallergic activity and that a polar functional group such as hydroxymethyl present on the ethyl chain of the ethanolamide moiety yields better fatty acid derivatives with improved activity over DHEA. Moreover, a longer alkyl chain of alcohol functionalities such as propanol and diethylene glycol than that of the ethanol of the ethanolamide moiety is highly effective for making better fatty acids of improved potency.

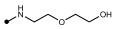
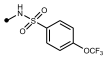
Next, in order to see whether improved antiallergic activity can be gained by other substituents, we attempted to switch the ethanolamide moiety of DHEA with a series of substituted aryls (**37**−**39**), cyclolalkyls (**40**−**43**), and sulfones (**44**−**50**) as listed in Table 3. Because amide functionality and a primary alcohol were found to be useful to increase antiallergic activity as indicated in Table 2, amide derivatives with one or two OH groups on the benzene ring were synthesized. As observed in compounds **37**−**39**, no activity was yielded in hydroxylated arylamide derivatives in contrast to the results obtained from hydroxyalkylamide compounds (**28**, **29**, **34**, and **35** in Table 2), implying that OH groups present on the benzene ring are not useful for producing antiallergic activity. In addition, no significant potency was induced by the addition of heterocycloalkylamide derivatives with morpholine (**40**), hydropyran (**41** and **42**), methylpiperazine (**43**), or methylsulfonylpiperazine (**44**), showing that heterocycloalkyls are not effective for potency, as well. Methylsulfonamide (**45**) missing piperazine from compound **26** did not make any change in activity. However, interestingly, replacing the methyl group of the methylsulfonamide moiety of compound **45** with a *tert*-butyl (**46**) resulted in a dramatic increase in potency, implying that a *tert*-butyl group attached on sulfonamide is a highly effective substituent to give potent activity. Furthermore, it was implied that substituted sulfonamide is a useful functionality to improve antiallergic activity of DHEA. When the sulfonyl group of compound **46** was eliminated to see whether it is necessary for inducing activity, no meaningful potency was observed as seen in compound **47**, proving that the polar sulfonyl function of the *tert*-butylsulfonamide moiety of compound **46** plays a critical role in the generation of activity. In order to further optimize compound **46**, the replacement of the *tert*-butyl of the *tert*-butylsulfonamide moiety of compound **46** was tried by a cyclopropyl (**48**), a trifluoromethyl (**49**), or a pentafluoroethyl (**50**). No significant or more than 2-fold decreased activity was observed in cyclopropyl (**48**) and trifluoromethyl (**49**) derivatives, respectively, whereas a little reduced potency compared to that of compound **46** was gained from pentafluoroethylamide compound (**50**). This indicates that a *tert*-butyl group is the most promising function among other alkyl substituents tested for the sulfonamide moiety to make improved fatty acid derivatives with enhanced antiallergic activity. The fatty acid attached with *tert*-butylsulfonamide (**46**) had better antiallergic activity than DHEA, which was similar to that of hydroxyalkylamide derivatives (**28**, **29**, **34**, and **35** in Table 2). In addition, it was found that hydroxyarylamides and heterocycloalkylamides are not useful for producing promising fatty acid derivatives.

In order to further optimize the sulfonamide function, a series of aryls were incorporated to the sulfonamide moiety instead of the *tert*-butyl group of compound **46** in Table 3. As shown in Table 4, fatty acid derivatives with substituted (**53**−**61**) or nonsubstituted (**51**, **52**, and **62**) arylsulfonamide were synthesized and evaluated for anti-degranulating activity in the mast cells. As seen in compound **51**, sulfonamide with a phenyl group gave no activity, whereas the replacement of the phenyl group of compound **51** by a benzyl (**52**) dramatically increased activity. When incorporating a methyl group on the benzene ring of compound **51** (**53**), improved potency was also gained, which is similar to that of DHEA. These results indicate that one carbon chain present between the sulfonyl and the benzene ring (**52**) or on the benzene ring (**53**) in the arylsulfonamide moiety is highly effective for producing significant activity. On the other hand, in compound **54** with a hydroxy on the benzene ring instead of the methyl group of compound **53**, no activity was observed, implying that a hydroxy group on the aryl moiety is not useful for affording relevant activity in arylsulfonamide derivatives. This was a similar result to that found in compounds with hydroxyphenylamide derivatives (**37**−**39** in Table 3). However, it was contrast to the result that hydroxyalkylamide compounds (**28**, **29**, **34**, and **35** in Table 2) exhibited very potent activity. Substituting a fluoro (**55**) or a chloro (**56**) group on the benzene ring led to similar potency to that of DHEA, which implies that a halogen atom added to the benzene ring of the arylsulfonamide moiety is effective for retaining antiallergic activity comparable to that of DHEA. Furthermore, the presence of a trifluoromethyl (**57**) or a trifluoromethoxy (**58**) group on the benzene ring provided further enhanced activity, suggesting that the incorporation of halogen atom-containing substituents on the benzene ring is necessary to yield very potent arylsulfonamide fatty acid derivatives. Interestingly, compound with a nitro group on the benzene ring (**59**) had very potent


DOI: 10.1021/acs.jmedchem.9b00994
J. Med. Chem. 2019, 62, 9576−9592

**Table 5. PCA Suppressive Activity of DHEA Derivatives in Mice**



| No. | R | $IC_{50}$ (μM) [a] | PCA suppression (%) [b] | Log P [c] | FAAH substrate | FAAH inhibition |
|---|---|---|---|---|---|---|
| **DHA** | | >50 | 6 | 6.36 | ND [d] | ND |
| **12** | Ethanolamide of α-linolenic acid | 29 | 29 | 4.72 | ND | ND |
| **DHEA** | | 25 | 31 | 5.43 | Yes [e] | No [f] |
| **26** | | 24 | 7 | 5.75 | No [g] | No |
| **28** | | 16 | 17 | 4.89 | No | No |
| **29** | | 17 | 23 | 4.89 | Yes | No |
| **32** | | 24 | 0 | 5.21 | Yes | 8.6 [h] |
| **34** | | 18 | 0 | 5.53 | Yes | No |
| **35** |  | 18 | 11 | 5.27 | Yes | No |
| **46** | | 17 | 0 | 6.37 | No | No |
| **52** | | 22 | 2 | 6.88 | Yes | No |
| **55** | | 24 | 0 | 7.32 | Yes | No |
| **57** | | 15 | 19 | 8.08 | Yes | No |
| **58** |  | 19 | 11 | 8.6 | Yes | No |
| **59** | | 15 | 49* | 5.47 | No | 36 [h] |
| | Fexofenadine | ND | 39 [i] | ND | ND | ND |

[a]Anti-degranulating activity was evaluated by β-hexosaminidase release assay. [b]Suppression of PCA reaction (%) relative to the control group at 1000 mg/kg. Data are expressed as the mean of each group. [c]log *P*: calculated log *P* by Crippen's method by using CS ChemDraw 6.0 version. [d]Not determined. [e]The peak of DHA was detected by HPLC analysis after the incubation of each compound with the human FAAH. [f]No FAAH inhibition activity when $IC_{50}$ is over 50 μM. [g]The peak of DHA was not detectable after the incubation of each compound with the human FAAH. [h]$IC_{50}$ (μM). [i]Suppression of PCA reaction (%) relative to the control group at 50 mg/kg. Fexofenadine is a histamine $H_1$ antagonist ($K_i$ = 10 nM). *$P < 0.05$ by Dunnett's test.

antiallergic activity, as well, implying that a strong electron-withdrawing group on the aryl moiety is highly effective to generate promising arylsulfonamide derivatives. On the other hand, in the benzoic acid derivative (**60**), no activity was gained, but the corresponding methyl benzoate (**61**) showed significantly improved activity, which indicates that an ester function is more effective than the corresponding acid in arylsulfonamide derivatives for inducing activity. This was different from the result shown in Table 2 that free acid function (**32**) is better for activity over the corresponding ester (**33**) in alkylamide derivatives. The compound attached with thiophenesulfonamide (**62**) did not lead to meaningful potency, implying that the addition of a heteroaryl substituent to the arylsulfonamide moiety does not work for making relevant potency. It was found in Table 4 that arylsulfonamides with a substituent such as a methyl or a halogen atom produce potent fatty acid derivatives comparable to DHEA. Moreover, substituents, such as a trifluoromethyl, a trifluoromethoxy, and a nitro group, on the aryl moiety of arylsulfonamide derivatives are highly effective for yielding very potent antiallergic activity.

DHEA derivatives with promising in vitro antiallergic activity were found as shown in Tables 2−4. Among them, twelve compounds with better potency than that of DHEA were selected and then used for the evaluation of in vivo antiallergic activity by using an IgE-dependent passive cutaneous anaphylaxis (PCA) reaction in mice.[31−33] Because PCA is a localized cutaneous allergic response caused by an allergen exposure, PCA suppressive activity is widely used for assessing the in vivo antiallergic effect of bioactive compounds.[32,33] As shown in Table 5, orally administered DHEA suppressed PCA reaction in mice about 30%, whereas almost no suppressive effect on PCA reaction was observed in DHA treated mice, suggesting that DHEA with antiallergic activity in vitro is effective for attenuating an allergic response in vivo, as well. It was also indicated that in vivo antiallergic activity observed by the treatment of DHEA is induced by DHEA in mice not by


DOI: 10.1021/acs.jmedchem.9b00994
J. Med. Chem. 2019, 62, 9576−9592

other bioactive fatty acids converted from DHA, a metabolite of DHEA, because DHA treatment led to very low PCA suppression in mice in this study. On the other hand, studies indicate that DHEA is also oxygenated to a range of bioactive metabolites effective for the treatment of inflammatory and neurological disorders.[34−36] When ethanolamide of linolenic acid (**12**) with a similar in vitro activity to that of DHEA was examined for in vivo potency, almost the same result as that of DHEA was gained, indicating that a difference in the length of the lipid part of DHEA and compound **12** does not affect in vivo potency. However, substituted alkylamide derivatives with better in vitro antiallergic activity (**26**, **28**, **29**, **32**, **34**, and **35**) than that of DHEA did not exert increased in vivo activity over that of DHEA. Compounds **26**, **28**, **29**, and **35** made 7−23% suppression in PCA reaction and compounds **32** and **34** did not provide any suppressive effect. Similar results were observed in substituted sulfonamide derivatives (**46**, **52**, **55**, **57**, and **58**), which showed 0−19% suppressive activity of PCA reaction. Although the test compounds were more potent than DHEA in vitro, they were less potent in vivo. However, in compound **59** with 4-nitrophenylsulfonamide, approximately 50% suppressive activity of PCA reaction was gained, which was 20% higher in comparison to the result of DHEA. However, it was approximately 150-fold less potent compared to that of a positive control (fexofenadine) with antiallergic activity, meaning that further structural optimizations need to be conducted to develop compounds with better potency in vitro and in vivo in the future research. In this study, only compound **59** showed expected in vivo result corresponding to the in vitro potency. There are many factors that can influence bioavailability. For example, undesirable physical properties of compounds such as high lipophilicity or low water solubility can lead to reduced bioactivity.[21] When lipophilicity (log *P*) of test compounds in Table 5 was calculated, substituted alkylamide derivatives had similar lipophilicity to that of DHEA. On the other hand, much higher lipophilicity than that of DHEA was observed in substituted sulfonamide compounds except compound **59**. Interestingly, lipophilicity of compound **59** was almost same as that of DHEA, implying that lipophilicity can be one of the important factors to affect in vivo activity of the fatty acid derivatives. On the other hand, DHEA is reported as a substrate of fatty acid amide hydrolase (FAAH),[37] which degrades DHEA into DHA and 2-aminoethanol. Thus, hydrolysis of DHEA and its derivatives by FAAH can result in a loss of activity in vivo by producing less potent DHA. In addition, it is reported that cellular uptake of ethanolamide of arachidonic acid (**14**) is affected by the FAAH expression level,[38] indicating that the hydrolyzing enzyme may play a key role in the modulation of in vivo activity of DHEA and fatty acid derivatives. We thus investigated whether or not the fatty acids interact with human FAAH. As shown in Table 5, as expected, DHEA was hydrolyzed by the human FAAH to less potent DHA. Most of the derivatives in Table 5 were also hydrolyzed to DHA by the enzyme activity except compounds **26**, **28**, **46**, and **59**. Although compounds **26**, **28**, and **46** were not substrates for the human FAAH, lower in vivo potency than that of DHEA was exerted. Only compound **59** that is not a substrate of the human FAAH had better potency compared to that of DHEA, indicating that there is no reasonable relationship between hydrolysis by the human FAAH and in vivo antiallergic activity and that FAAH-mediated cellular uptake does not affect in vivo potency of compound **59**. Furthermore, when inhibition of the human FAAH by DHEA and fatty acid derivatives was examined, most of compounds in Table 5 did not inhibit FAAH activity except compounds **32** and **59**. Compounds with no FAAH inhibition activity had decreased antiallergic activity in vivo compared to that of DHEA. In the case of compound **32** with FAAH inhibition potency, it seems that it acts as a substrate for the enzyme with a higher affinity rather than behaving as an inhibitor of the human FAAH, resulting in no antiallergic activity in vivo. Interestingly, compound **59**, which is not a FAAH substrate, was found as a potent inhibitor of the enzyme. This result implies that the ability of FAAH inhibition of compound **59** is one of critical properties to make it a potent compound with high antiallergic activity in vivo over DHEA. Surprisingly, when DHEA was co-treated with a FAAH inhibitor, in vitro antiallergic activity of DHEA was improved about 3-fold compared to that without an inhibitor (Table 6), again suggesting that suppressing FAAH activity is highly important to produce enhanced antiallergic activity in vitro and in vivo. Compounds with a dual action of anti-degranulation activity and FAAH inhibition activity will be highly promising for the effective treatment of allergy. Overall results in Tables 5 and 6 indicate that a physical property such as lipophilicity and a biochemical property such as inhibition of the human FAAH are closely related to in vivo antiallergic activity of the fatty acid derivatives, whereas no direct relationship with hydrolysis by the human FAAH is observed.

**Table 6. Anti-degranulating Activity of DHEA and DHEA Co-Treated with URB597 (a FAAH Inhibitor) or AM630 (a CB2 Antagonist) in RBL-2H3 Cells**

| no. | $IC_{50}$ ($\mu$M)[a] |
|---|---|
| DHEA | 25 |
| DHEA + URB597 | 7.6 |
| DHEA + AM630 | 25 |

[a]The activity was evaluated by a $\beta$-hexosaminidase release assay.

DHEA is known to act via cannabinoid receptor 1 (CB1) and CB2 when it exerts its biological functions.[37] Because RBL-2H3 cells used in this study express CB2, but not CB1,[39] the effect of CB2 antagonism on anti-degranulation activity of DHEA was investigated. When anti-dinitrophenol (DNP) IgE-sensitized RBL-2H3 cells were treated with DHEA in the presence of 10 $\mu$M AM630 (a CB2 antagonist), almost same anti-degranulation result as that of the cells treated with DHEA only was observed (Table 6). This suggests that DHEA does not interact with CB2 when it shows its anti-degranulation activity in vitro. Recently, it has been reported that DHEA interacts with a receptor called GPR110 expressed on nerve cells,[40] showing that this may be a potential target molecule that DHEA binds to in exerting the antiallergic effect. It will be valuable to investigate the relationship between anti-degranulation activity of fatty acid derivatives and GPR110 to elucidate a physiological target of the fatty acids in the future research. In the present study, we found improved DHEA derivatives both in vitro and in vivo by using extensive SAR studies, but it was also implied that further optimizations are necessary for developing improved compounds in vitro and in vivo. The SAR data established through this study will be a highly useful base for the future research.

## ■ CONCLUSIONS

This report focused on investigating SARs of unsaturated fatty acid derivatives toward developing novel fatty acids with promising antiallergic activity in vitro and in vivo. First, in order to find fatty acid components with antiallergic activity in


DOI: 10.1021/acs.jmedchem.9b00994
J. Med. Chem. 2019, 62, 9576−9592

fish oil, a series of unsaturated fatty acids present in fish oil and their corresponding ethanolamide metabolites were prepared and assessed for anti-degranulating activity in the mast cells as an anti-allergic activity (Table 1). The data indicated that ethanolamides of three omega-3 fatty acids (**12**, **15**, and **18**) are effective for the suppression of allergic reaction in the cells. However, the corresponding free omega-3 fatty acids (**3**, $\alpha$-linolenic acid; **6**, EPA; **9**, DHA) themselves were found not to have antiallergic activity. Other omega-6 or -9 fatty acids and their corresponding ethanolamides did not show significant potency, either. These results suggest that the converted ethanolamide metabolites of the omega-3 fatty acids are active ingredients to exert antiallergic activity in the body. Based on this finding, extensive SAR studies were carried out by using DHA-ethanolamide (DHEA) as a lead structure to develop fatty acid derivatives with improved antiallergic activity in vitro and in vivo (Tables 2−5). When the ethanolamide moiety of DHEA was modified by a variety of substituted alkylamides (Table 2), improved fatty acids with better antiallergic activity over that of DHEA were produced by the incorporation of a polar hydroxymethyl group to the ethyl chain of the moiety (**34** and **35**). However, in compounds with a methyl substituent on the ethyl chain (**26** and **27**), NH (**23** and **25**), or OH (**24**) of the ethanolamide moiety, decreased or no activity was gained. The corresponding ester analogue (**22**) of DHEA possessed no activity, suggesting that the nonsubstituted amide functionality and polar group incorporation are structurally required to yield enhanced activity in alkylamide derivatives. Furthermore, longer alcohol functions (**34** and **35**) than ethanol of the moiety were useful for making promising fatty acid derivatives. Next, a variety of substituted arylamides (**37**−**39**), cycloalkylamides (**40**−**43**), and sulfonamides (**44**−**50**) were synthesized (Table 3). The results showed that a *tert*-butylsulfonamide group (**46**) present in the place of the ethanolamide moiety of DHEA is a highly promising function for yielding potent antiallergic activity over that of DHEA. However, the replacement of the *tert*-butyl group of compound **46** with a methyl (**45**) or other functions (**48**−**50**) dropped activity. In addition, no activity was observed in substituted arylamides (**37**−**39**) and cycloalkylamides (**40**−**43**), suggesting that alkylsulfonamide is a novel functionality effective for the production of fatty acids with improved antiallergic activity (Table 3). In order to further optimize the sulfonamide function, a series of substituted arylsulfonamide derivatives were synthesized (Table 4). Interestingly, compounds with a trifluoromethyl (**57**), a trifluoromethoxy (**58**), or a nitro (**59**) group on the benzene ring of the arylsulfonamide function exhibited very potent activity comparable to that of compound **46**. In replacing a nitro group on the benzene ring of compound **59** by a methyl (**53**) or a halogen atom (**55**−**56**), decreased activity was observed. A benzyl (**52**) or benzoate (**61**) induced a reduction in activity, as well. In other substituents (**51**, **54**, **60**, and **62**), no antiallergic activity was seen, suggesting that a halogenated alkyl (**57**−**58**) or a strong electron withdrawing group (**59**) on the benzene ring of the arylsulfonamide moiety is a highly effective substituent to yield promising fatty acid derivatives better than DHEA. In the present study, significant DHEA derivatives with antiallergic activity were found through extensive SAR studies as shown in Tables 2−4. Among them, twelve compounds with better in vitro antiallergic activity than that of DHEA were used to investigate suppressive activity of PCA reaction in mice as an in vivo antiallergic activity (Table 5). The result indicated that DHEA has approximately 30% suppressive activity of PCA reaction in mice, whereas no suppression is gained by the treatment of DHA with no in vitro antiallergic activity, suggesting that compounds with antiallergic activity in vitro exhibit reasonable activity in vivo, as well. When twelve compounds were orally administered to mice, only compound **59** with nitrophenylsulfonamide showed approximately 20% increased PCA suppressive activity in mice compared to that of DHEA. Other compounds had decreased or no in vivo activity although they were more potent in vitro than DHEA. When lipophilicity of test compounds was calculated, compound **59** showed almost same hydrophobicity as that of DHEA, whereas much higher hydrophobility was obtained from other arylsulfonamide derivatives, implying that lipophilicity of fatty acid derivatives may be one of the important factors that affect in vivo availability. In addition, compound **59** with an amide function was not hydrolyzed by the human FAAH, whereas DHEA and most of fatty acid derivatives were subjected to hydrolysis by the enzyme. Interestingly, even compound **59** inhibited activity of the human FAAH, suggesting that the biochemical property of compound **59** is a critical factor to make it a potent antiallergic agent in vivo compared to DHEA and other derivatives. A significant improvement in in vitro antiallergic activity of DHEA with a co-treatment of a FAAH inhibitor further supported the importance of FAAH inhibition by fatty acid derivatives for producing potent antiallergic activity in vivo. In this study, valuable fatty acid derivatives with enhanced activity in vitro and in vivo were found, and they will be important lead structures for further development of novel fatty acids with improved in vitro and in vivo activity. Moreover, the resulting compounds will be useful tools for investigating DHA- or DHEA-related biological functions and treating the disorders associated with DHEAsuch as allergy.

## ■ EXPERIMENTAL SECTION

**Chemistry.** Unless otherwise noted, all materials were purchased from commercial suppliers and used without further purification. $^1$H NMR and $^{13}$C NMR spectra were recorded on a JEOL JNM-EX400 spectrometer (JEOL, Japan) using tetramethylsilane as an internal standard. High-resolution mass spectra (HRMS) were recorded on a Xevo quadrupole time-of-flight mass spectrometer (Waters, Milford, MA, USA) in the positive/negative ion mode. Thin-layer chromatography was performed on EMD precoated silica gel 60 F254 plates, and spots were visualized with UV light and stained with basic $KMnO_4$. Synthetic methods are described for representative compounds. Based on the HPLC analysis described below, the purity of all final compounds was determined to be greater than 95% unless otherwise indicated. HPLC purity analysis was conducted on an Acquity UPLC system (Waters) with a diode array detector. Chromatographic separations were conducted using an Acquity UPLC BEH $C_{18}$ (1.7 $\mu$m, 2.1 × 100 mm) column (Waters). A flow rate of 0.4 mL/min was used with an isocratic mobile phase of 95% acetonitrile. The absorbance was detected at 210 nm.

*(9Z,12Z,15Z)-N-(2-Hydroxyethyl)octadeca-9,12,15-trienamide (**12**).* To a solution of $\alpha$-linoleic acid (0.20 g, 0.71 mmol), DMAP (0.11 g, 0.86 mmol) and 2-aminoethanol (88 mg, 1.43 mmol) in dry dichloromethane (5 mL) were added EDCI (0.17 g, 0.86 mmol) at room temperature. The reaction was stirred overnight under nitrogen gas. The product was extracted with ethyl acetate (50 mL × 2). The combined organic solutions were washed with an aqueous solution of 1 N hydrochloric acid (15 mL) and brine (70 mL × 2), dried over magnesium sulfate, and evaporated to dryness. The residue was purified by using silica gel column chromatography (hexane/ethyl acetate = 2:1) to afford compound **12** in 78% yield. $^1$H NMR: $\delta$ ($CDCl_3$) 0.97 (3H, t, $J$ = 7.2 Hz), 1.25−1.40 (8H, m), 1.59−1.66 (2H, m), 2.02−2.11 (4H, m), 2.20 (2H, t, $J$ = 7.2 Hz), 2.76−2.83 (4H, m), 2.99 (1H, s), 3.42 (2H, q, $J$ = 7.2 Hz), 3.72 (2H, t, $J$ = 7.2 Hz), 5.29−5.42 (6H, m), 6.05 (1H, s). $^{13}$C NMR: $\delta$ ($CDCl_3$) 14.26, 20.51, 25.49, 25.58, 25.67, 27.16,


DOI: 10.1021/acs.jmedchem.9b00994
J. Med. Chem. 2019, 62, 9576−9592

29.09, 29.22, 29.55, 36.63, 42.40, 62.51, 127.06, 127.69, 128.20, 128.24, 130.22, 131.94, 174.57. HRMS (ESI) $m/z$: calcd for $C_{20}H_{35}NO_2$ [M + H]$^+$, 322.2746; found [M + H]$^+$, 322.2748.

Compounds **13** and **17** were synthesized in the same procedure performed for the preparation of compound **12** by using a corresponding fatty acid instead of α-linoleic acid.

*(8Z,11Z,14Z)-N-(2-Hydroxyethyl)icosa-8,11,14-trienamide (**13**).* $^1$H NMR: δ ($CDCl_3$) 0.89 (3H, t, $J$ = 7.2 Hz), 1.23−1.39 (12H, m), 1.61−1.66 (2H, m), 2.05 (4H, quint, $J$ = 7.2 Hz), 2.21 (2H, t, $J$ = 7.2 Hz), 2.64 (1H, t, $J$ = 7.2 Hz), 2.81 (4H, t, $J$ = 7.2 Hz), 3.43 (2H, q, $J$ = 7.2 Hz), 3.73 (2H, q, $J$ = 7.2 Hz), 5.30−5.43 (6H, m), 5.90 (1H, s). $^{13}$C NMR: δ ($CDCl_3$) 14.08, 22.56, 25.60, 27.14, 27.20, 28.96, 29.31, 29.44, 31.51, 36.62, 42.48, 62.75, 127.59, 127.81, 128.18, 128.30, 130.12, 130.43, 174.48. HRMS (ESI) $m/z$: calcd for $C_{22}H_{39}NO_2$ [M + H]$^+$, 350.3059; found [M + H]$^+$, 350.3090.

*(13Z,16Z)-N-(2-Hydroxyethyl)docosa-13,16-dienamide (**17**).* $^1$H NMR: δ ($CDCl_3$) 0.89 (3H, t, $J$ = 7.2 Hz), 1.26−1.39 (22H, m), 1.61−1.65 (2H, m), 2.05 (4H, quint, $J$ = 7.2 Hz), 2.20 (2H, t, $J$ = 7.2 Hz), 2.60 (1H, t, $J$ = 7.2 Hz), 2.77 (2H, t, $J$ = 7.2 Hz), 3.43 (2H, q, $J$ = 7.2 Hz), 3.73 (2H, q, $J$ = 7.2 Hz), 5.29−5.41 (4H, m), 5.88 (1H, s). $^{13}$C NMR: δ ($CDCl_3$) 14.08, 25.60, 25.69, 27.18, 29.33, 29.47, 29.53, 29.59, 29.67, 36.67, 42.50, 62.81, 127.91, 130.17, 174.56. HRMS (ESI) $m/z$: calcd for $C_{24}H_{45}NO_2$ [M + H]$^+$, 380.3529; found [M + H]$^+$, 380.3565.

*(4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-Hydroxyethyl)docosa-4,7,10,13,16,19-hexaenamide (**18**, DHEA).* To a solution of DHA (0.30 g, 0.91 mmol), DMAP (0.13 g, 1.09 mmol) and 2-aminoethanol (0.082 g, 1.09 mmol) in dry dichloromethane (7 mL) were added EDCI (0.21 g, 1.09 mmol) at room temperature. The reaction mixture was stirred overnight at room temperature under nitrogen gas. The product was extracted with ethyl acetate (50 mL × 2). The combined organic solutions were washed with an aqueous solution of 1 N hydrochloric acid (15 mL) and brine (70 mL × 2), dried over magnesium sulfate, and evaporated to dryness. The residue was purified by using silica gel column chromatography eluting ethyl acetate to afford **18** (DHEA) in 92% yield. $^1$H NMR: δ ($CDCl_3$) 0.97 (3H, t, $J$ = 7.2 Hz), 2.07 (2H, quint, $J$ = 7.2 Hz), 2.27 (2H, t, $J$ = 7.2 Hz), 2.42 (2H, q, $J$ = 7.2 Hz), 2.65 (1H, s), 2.80−2.88 (10H, m), 3.43 (2H, q, $J$ = 7.2 Hz), 3.72 (2H, t, $J$ = 7.2 Hz), 5.25−5.44 (12H, m), 5.95 (1H, s). $^{13}$C NMR: δ ($CDCl_3$) 14.27, 20.53, 23.35, 25.51, 25.60, 36.30, 42.47, 62.56, 126.97, 127.83, 127.97, 128.03, 128.25, 128.30, 128.55, 129.46, 132.03, 173.64. HRMS (ESI) $m/z$: calcd for $C_{24}H_{37}NO_2$ [M + H]$^+$, 372.2903; found [M + H]$^+$, 372.2919.

Compounds **20**−**23**, **26**−**30**, **34**−**61**, and **62** were synthesized in the same manner used for the preparation of DHEA by using a corresponding amine or ester instead of 2-aminoethanol.

*(4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-Aminoethyl)docosa-4,7,10,13,16,19-hexaenamide (**19**).* To a solution of DHA (0.10 g, 0.30 mmol), DMAP (37 mg, 0.30 mmol) and *tert*-butyl 2-aminoethylcarbamate (48 mg, 0.30 mmol) in dry dichloromethane (7 mL) were added EDCI (58 mg, 0.30 mmol) at room temperature. The reaction mixture was stirred overnight at room temperature under nitrogen gas. The product was extracted with ethyl acetate (50 mL × 2). The combined organic solutions were washed with an aqueous solution of 1 N hydrochloric acid (15 mL) and brine (70 mL × 2), dried over magnesium sulfate, and evaporated to dryness. To the residue dissolved in dichloromethane (5 mL) was added trifluoroacetic acid (1 mL). The reaction mixture was stirred for 1 h at room temperature. Triethylamine (2 mL) was then added to the reaction mixture at 0 °C, and the reaction was stirred for 10 min. The product was extracted with ethyl acetate (50 mL × 2). The combined organic solutions were washed with brine (70 mL × 2), dried over magnesium sulfate, and evaporated to dryness. The residue was purified by using silica gel column chromatography (hexane/acetone = 2:1) to afford compound **19** in 57% yield. $^1$H NMR: δ ($CDCl_3$) 0.97 (3H, t, $J$ = 7.2 Hz), 1.25 (2H, s), 2.07 (2H, quint, $J$ = 7.2 Hz), 2.26 (2H, t, $J$ = 7.2 Hz), 2.40 (2H, q, $J$ = 7.2 Hz), 2.79−2.94 (12H, m), 3.35 (2H, s), 5.27−5.43 (12H, m), 6.36 (1H, s). $^{13}$C NMR: δ ($CDCl_3$) 14.26, 20.53, 23.37, 25.50, 25.59, 29.68, 36.37, 40.94, 126.97, 127.83, 128.04, 128.22, 128.54, 129.24, 132.01, 172.97. HRMS (ESI) $m/z$: calcd for $C_{24}H_{38}N_2O$ [M + H]$^+$, 371.3062; found [M + H]$^+$, 371.3091.

*(4Z,7Z,10Z,13Z,16Z,19Z)-N-Propyldocosa-4,7,10,13,16,19-hexaenamide (**20**).* $^1$H NMR: δ ($CDCl_3$) 0.91−0.99 (6H, m), 1.51 (2H, sext, $J$ = 7.2 Hz), 2.07 (2H, quint, $J$ = 7.2 Hz), 2.22 (2H, t, $J$ = 7.2 Hz), 2.41 (2H, q, $J$ = 7.2 Hz), 2.80−2.84 (10H, m), 3.21 (2H, q, $J$ = 7.2 Hz), 5.26−5.42 (13H, m). $^{13}$C NMR: δ ($CDCl_3$) 11.35, 14.26, 20.53, 22.87, 23.46, 25.50, 25.60, 36.54, 41.18, 126.97, 127.83, 128.03, 128.22, 128.30, 128.54, 129.24, 132.02, 172.22. HRMS (ESI) $m/z$: calcd for $C_{25}H_{39}NO$ [M + H]$^+$, 370.3110; [M + H]$^+$, 370.3137.

*(4Z,7Z,10Z,13Z,16Z,19Z)-N-Isopropyldocosa-4,7,10,13,16,19-hexaenamide (**21**).* $^1$H NMR: δ ($CDCl_3$) 0.97 (3H, t, $J$ = 7.2 Hz), 1.14 (6H, d, $J$ = 7.2 Hz), 2.07 (2H, quint, $J$ = 7.2 Hz), 2.18 (2H, t, $J$ = 7.2 Hz), 2.40 (2H, q, $J$ = 7.2 Hz), 2.80−2.86 (10H, m), 4.03−4.12 (1H, m), 5.28−5.44 (13H, m). $^{13}$C NMR: δ ($CDCl_3$) 14.26, 20.53, 22.82, 23.46, 25.51, 25.59, 36.63, 41.26, 126.98, 127.84, 128.07, 128.23, 128.31, 128.55, 129.19, 132.02, 171.32. HRMS (ESI) $m/z$: calcd for $C_{25}H_{39}NO$ [M + H]$^+$, 370.3110; found [M + H]$^+$, 370.3129.

*(4Z,7Z,10Z,13Z,16Z,19Z)-2-Hydroxyethyl Docosa-4,7,10,13,16,19-hexaenoate (**22**).* $^1$H NMR: δ ($CDCl_3$) 0.97 (3H, t, $J$ = 7.2 Hz), 2.07 (2H, quint, $J$ = 7.2 Hz), 2.40−2.44 (4H, m), 2.80−2.88 (10H, m), 3.80−3.84 (2H, m), 4.21−4.23 (2H, m), 5.25−5.45 (12H, m). $^{13}$C NMR: δ ($CDCl_3$) 14.26, 20.53, 22.72, 25.50, 25.59, 34.03, 61.25, 66.03, 126.97, 127.75, 127.83, 127.96, 128.04, 128.22, 128.24, 128.28, 128.54, 129.43, 132.02, 173.41. HRMS (ESI) $m/z$: calcd for $C_{24}H_{36}O_3$ [M + H]$^+$, 373.2743; found [M + H]$^+$, 373.2796.

*(4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-Hydroxyethyl)-N-methyldocosa-4,7,10,13,16,19-hexaenamide (**23**).* $^1$H NMR: δ ($CDCl_3$) 0.97 (3H, t, $J$ = 7.2 Hz), 2.07 (2H, quint, $J$ = 7.2 Hz), 2.37−2.47 (4H, m), 2.80−2.88 (10H, m), 3.06 (3H, s), 3.56 (2H, t, $J$ = 7.2 Hz), 3.78 (2H, t, $J$ = 7.2 Hz), 5.30−5.42 (12H, m). $^{13}$C NMR: δ ($CDCl_3$) 14.26, 20.53, 22.78, 25.50, 25.60, 33.37, 36.80, 51.57, 61.84, 126.98, 127.84, 128.12, 128.22, 128.40, 128.53, 128.82, 129.04, 132.01, 174.59. HRMS (ESI) $m/z$: calcd for $C_{25}H_{39}NO_2$ [M + H]$^+$, 386.3059; found [M + H]$^+$, 386.3081.

*(4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-Methoxyethyl)docosa-4,7,10,13,16,19-hexaenamide (**24**).* To a solution of di-*tert*-butyldicarbonate (5.35 g, 24.5 mmol) in dichloromethane (7 mL) was added 2-aminoethanol (1.50 g, 24.5 mmol) at 0 °C. The reaction was stirred overnight at room temperature. The product was extracted with ethyl acetate (70 mL × 4). The combined organic solutions were washed with water (70 mL), dried over magnesium sulfate, and evaporated to dryness to give *tert*-butyl 2-hydroxyethylcarbamate in 95% yield. To a suspension of sodium hydride (0.21 g, 5.21 mmol) in dry DMF (5 mL) was added the protected aminoethanol (0.70 g, 4.34 mmol) in dry DMF (1 mL) at 0 °C. After stirring for 5 min, iodomethane (0.74 g, 5.21 mmol) in DMF (1 mL) was added to the reaction. The reaction mixture was stirred overnight at room temperature, and water (15 mL) was added to the reaction to quench the reactivity of remained sodium hydride. The product was extracted with diethyl ether (70 mL × 2). The organic layer was washed with water (70 mL × 2), dried over magnesium sulfate, and evaporated to dryness. The residue was purified by using silica gel column chromatography (hexane/ethyl acetate = 5:1) to afford *tert*-butyl 2-methoxyethylcarbamate in 87% yield.

To a solution of the above-protected amine intermediate (0.16 g, 0.91 mmol) in dichloromethane (5 mL) was added trifluoroacetic acid (1.04 g, 9.13 mmol) at room temperature. The reaction was stirred overnight and triethylamine (1.07 g, 10.6 mmol) was then added to the reaction mixture at 0 °C. After stirring for 15 min at this temperature, DHA (0.25 g, 0.76 mmol), DMAP (0.11 g, 0.91 mmol), and EDCI (0.18 g, 0.91 mmol) were added to the reaction mixture at room temperature. The reaction was stirred overnight under nitrogen gas. The product was extracted with ethyl acetate (70 mL × 2). The combined organic solutions were washed with an aqueous solution of 1 N hydrochloric acid (15 mL) and brine (70 mL × 2), dried over magnesium sulfate, and evaporated to dryness. The residue was purified by using silica gel column chromatography (hexane/ethyl acetate = 2:1) to afford compound **24** in 82% yield. $^1$H NMR: δ ($CDCl_3$) 0.97 (3H, t, $J$ = 7.2 Hz), 2.07 (2H, quint, $J$ = 7.2 Hz), 2.25 (2H, t, $J$ = 7.2 Hz), 2.41 (2H, q, $J$ = 7.2 Hz), 2.80−2.88 (10H, m), 3.35 (3H, s), 3.43−3.46 (4H, s), 5.25−5.44 (12H, m), 5.86 (1H, s). $^{13}$C NMR: δ ($CDCl_3$) 14.27, 20.53, 23.38, 25.51, 25.60, 36.39, 39.15, 58.74, 71.17, 126.98,


DOI: 10.1021/acs.jmedchem.9b00994
J. Med. Chem. 2019, 62, 9576−9592

127.84, 128.06, 128.15, 128.24, 128.54, 129.29, 132.02, 172.49. HRMS (ESI) $m/z$: calcd for $C_{25}H_{39}NO_2$ [M + H]$^+$, 386.3059; found [M + H]$^+$, 386.3102.

Compound **25** was synthesized in the same reactions used for the preparation of compound **24** by using 2-(methylamino)ethanol instead of 2-aminoethanol.

*(4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-Methoxyethyl)-N-methyldocosa-4,7,10,13,16,19-hexaenamide (**25**).* $^1$H NMR: $\delta$ ($CDCl_3$) 0.97 (3H, t, $J$ = 7.2 Hz), 2.07 (2H, quint, $J$ = 7.2 Hz), 2.35−2.44 (4H, m), 2.80−2.88 (10H, m), 3.06 (3H, s), 3.33 (3H, s), 3.48 (2H, s), 3.51−3.58 (2H, m), 5.25−5.45 (12H, m). $^{13}$C NMR: $\delta$ ($CDCl_3$) 14.27, 20.53, 22.88, 25.51, 25.59, 33.32, 37.04, 47.87, 58.77, 71.09, 126.99, 127.86, 128.07, 128.19, 128.22, 128.53, 128.59, 128.87, 132.01, 172.64. HRMS (ESI) $m/z$: calcd for $C_{26}H_{41}NO_2$ [M + H]$^+$, 400.3216; found [M + H]$^+$, 400.3217.

*(4Z,7Z,10Z,13Z,16Z,19Z)-N-(1-Hydroxypropan-2-yl)docosa-4,7,10,13,16,19-hexaenamide (**26**).* $^1$H NMR: $\delta$ ($CDCl_3$) 0.97 (3H, t, $J$ = 7.2 Hz), 1.17 (3H, d, $J$ = 7.2 Hz), 2.07 (2H, quint, $J$ = 7.2 Hz), 2.25 (2H, t, $J$ = 7.2 Hz), 2.41 (2H, q, $J$ = 7.2 Hz), 2.80−2.88 (10H, m), 3.50−3.55 (1H, m), 3.64−3.67 (1H, m), 4.02−4.11 (1H, m), 5.27−5.46 (12H, m), 5.63 (1H, s). $^{13}$C NMR: $\delta$ ($CDCl_3$) 14.27, 17.03, 20.54, 23.38, 25.51, 25.61, 36.43, 47.88, 67.37, 126.98, 127.84, 127.98, 128.04, 128.25, 128.32, 128.56, 129.46, 132.03, 173.16. HRMS (ESI) $m/z$: calcd for $C_{25}H_{39}NO_2$ [M + H]$^+$, 386.3059; found [M + H]$^+$, 386.3083.

*(4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-Hydroxypropyl)docosa-4,7,10,13,16,19-hexaenamide (**27**).* $^1$H NMR: $\delta$ ($CDCl_3$) 0.97 (3H, t, $J$ = 7.2 Hz), 1.18 (3H, d, $J$ = 7.2 Hz), 2.07 (2H, quint, $J$ = 7.2 Hz), 2.27 (2H, t, $J$ = 7.2 Hz), 2.42 (2H, q, $J$ = 7.2 Hz), 2.57 (1H, s), 2.80−2.88 (10H, m), 3.07−3.14 (1H, m), 3.41−3.47 (1H, m), 3.90−3.93 (1H, m), 5.27−5.46 (12H, m), 5.92 (1H, s). $^{13}$C NMR: $\delta$ ($CDCl_3$) 14.27, 20.54, 20.96, 23.39, 25.52, 25.61, 36.35, 47.05, 67.58, 126.98, 127.84, 127.98, 128.00, 128.07, 128.26, 128.29, 128.56, 129.45, 132.04, 173.48. HRMS (ESI) $m/z$: calcd for $C_{25}H_{39}NO_2$ [M + H]$^+$, 386.3059; found [M + H]$^+$, 386.3100.

*(4Z,7Z,10Z,13Z,16Z,19Z)-N-(1,3-Dihydroxypropan-2-yl)docosa-4,7,10,13,16,19-hexaenamide (**28**).* $^1$H NMR: $\delta$ ($CDCl_3$) 0.97 (3H, t, $J$ = 7.2 Hz), 2.07 (2H, quint, $J$ = 7.2 Hz), 2.30 (2H, t, $J$ = 7.2 Hz), 2.43 (2H, q, $J$ = 7.2 Hz), 2.64 (2H, s), 2.80−2.88 (10H, m), 3.72−3.87(4H, m), 3.92−3.97 (1H, m), 5.27−5.46 (12H, m), 6.22 (1H, s). $^{13}$C NMR: $\delta$ ($CDCl_3$) 14.28, 20.54, 23.38, 25.52, 25.61, 36.42, 52.26, 63.70, 126.98, 127.85, 127.98, 128.06, 128.25, 128.34, 128.56, 129.52, 132.04, 173.28. HRMS (ESI) $m/z$: calcd for $C_{25}H_{39}NO_3$ [M + H]$^+$, 402.3008; found [M + H]$^+$, 402.3035.

*(4Z,7Z,10Z,13Z,16Z,19Z)-N-(2,3-Dihydroxypropyl)docosa-4,7,10,13,16,19-hexaenamide (**29**).* $^1$H NMR: $\delta$ ($CDCl_3$) 0.97 (3H, t, $J$ = 7.2 Hz), 2.07 (2H, quint, $J$ = 7.2 Hz), 2.29 (2H, t, $J$ = 7.2 Hz), 2.42 (2H, q, $J$ = 7.2 Hz), 2.77−2.88 (10H, m), 3.00 (2H, s), 3.36−3.45 (2H, m), 3.51−3.58 (2H, m), 3.73−3.77 (1H, m), 5.27−5.47 (12H, m), 5.95 (1H, s). $^{13}$C NMR: $\delta$ ($CDCl_3$) 14.27, 20.54, 23.32, 25.52, 25.62, 36.23, 42.16, 63.45, 71.13, 126.99, 127.84, 127.90, 128.04, 128.28, 128.38, 128.58, 129.72, 132.05, 174.46. HRMS (ESI) $m/z$: calcd for $C_{25}H_{39}NO_3$ [M + H]$^+$, 402.3008; found [M + H]$^+$, 402.3040.

*(4Z,7Z,10Z,13Z,16Z,19Z)-N,N-Bis(2-hydroxyethyl)docosa-4,7,10,13,16,19-hexaenamide (**30**).* $^1$H NMR: $\delta$ ($CDCl_3$) 0.97 (3H, t, $J$ = 7.2 Hz), 2.07 (2H, quint, $J$ = 7.2 Hz), 2.40−2.48 (4H, m), 2.80−2.88 (10H, m), 3.50 (2H, t, $J$ = 7.2 Hz), 3.56 (2H, t, $J$ = 7.2 Hz), 3.78 (2H, t, $J$ = 7.2 Hz), 3.86 (2H, t, $J$ = 7.2 Hz), 5.25−5.44 (12H, m). $^{13}$C NMR: $\delta$ ($CDCl_3$) 14.26, 20.53, 22.99, 25.50, 25.59, 33.33, 50.52, 52.12, 60.85, 61.73, 126.98, 127.84, 128.06, 128.12, 128.21, 128.43, 128.54, 129.04, 132.02, 174.82. HRMS (ESI) $m/z$: calcd for $C_{26}H_{41}NO_3$ [M + H]$^+$, 416.3165; found [M + H]$^+$, 416.3202.

*(4Z,7Z,10Z,13Z,16Z,19Z)-1,3-Dihydroxypropan-2-yl Docosa-4,7,10,13,16,19-hexaenoate (**31**).* To a solution of glycerol (0.70 g, 7.60 mmol), DMAP (1.39 g, 11.4 mmol), and triethylamine (1.92 g, 19.0 mmol) in dichloromethane (15 mL) was added tert-butyldimethylchlorosilane (2.29 g, 15.2 mmol) in dichloromethane (5 mL). The reaction mixture was stirred overnight at room temperature. The product was extracted with diethyl ether (70 mL × 2). The organic layer was washed with an aqueous solution saturated with ammonium chloride (70 mL), an aqueous solution of 1 N hydrochloric acid (20 mL), and water (70 mL × 2), dried over magnesium sulfate, and evaporated to dryness. The residue was purified by using silica gel column chromatography (hexane/ethyl acetate = 2:1) to afford 1,3-protected glycerol in 50% yield.

To a solution of DHA (0.30 g, 0.91 mmol), DMAP (0.13 g, 1.09 mmol) and the above 1,3-protected glycerol (0.35 g, 1.09 mmol) in dry dichloromethane (7 mL) were added EDCI (0.21 g, 1.09 mmol) at room temperature. The reaction mixture was stirred overnight at room temperature under nitrogen gas. The product was extracted with ethyl acetate (50 mL × 2). The combined organic solutions were washed with an aqueous solution of 1 N hydrochloric acid (15 mL) and brine (70 mL × 2), dried over magnesium sulfate, and evaporated to dryness. The residue was purified by using silica gel column chromatography (hexane/ethyl acetate = 10:1) to afford DHA−glycerol ester intermediate in 82% yield.

1 N of tetrabutylammonium fluoride in THF (0.71 mL) was added to a solution of the glycerol ester intermediated (0.30 g, 0.47 mmol) in THF (15 mL) at 0 °C. The reaction was stirred overnight at room temperature. An aqueous solution saturated with ammonium chloride was added to the reaction (20 mL), and the reaction mixture was stirred for 5 min. The product was then extracted with ethyl acetate (70 mL × 2). The combined organic solutions were washed with brine (70 mL × 2), dried over magnesium sulfate, and evaporated to dryness. The residue was purified by using silica gel column chromatography (hexane/ethyl acetate = 2:1) to give compound **31** in 95% yield. $^1$H NMR: $\delta$ ($CDCl_3$) 0.97 (3H, t, $J$ = 7.2 Hz), 2.07 (2H, quint, $J$ = 7.2 Hz), 2.37−2.46 (4H, m), 2.80−2.88 (10H, m), 3.61−3.93 (4H, m), 4.13−4.23 (1H, m), 5.25−5.44 (12H, m). $^{13}$C NMR: $\delta$ ($CDCl_3$) 14.27, 20.54, 22.70, 25.51, 25.60, 34.00, 63.25, 65.28, 70.16, 126.98, 127.62, 127.84, 127.92, 128.04, 128.26, 128.33, 128.56, 129.57, 132.04, 173.54. HRMS (ESI) $m/z$: calcd for $C_{25}H_{38}O_4$ [M + H]$^+$, 403.2848; found [M + H]$^+$, 403.2868.

*2-((4Z,7Z,10Z,13Z,16Z,19Z)-Docosa-4,7,10,13,16,19-hexaenamido)acetic Acid (**32**).* To a solution of compound **33** (50 mg, 0.12 mmol) in methanol (10 mL) was added an aqueous solution of 1 N sodium hydroxide (3 mL). The reaction was stirred for 30 min at room temperature. After adjusting the pH to 9 by adding an aqueous solution of 1 N hydrochloric acid, the product was extracted with ethyl acetate (50 mL × 2). The combined organic solutions were washed with brine (70 mL × 2), dried over magnesium sulfate, and evaporated to dryness. The residue was purified by using silica gel column chromatography (100% ethyl acetate) to afford compound **32** in 98% yield. $^1$H NMR: $\delta$ ($CDCl_3$) 0.97 (3H, t, $J$ = 7.2 Hz), 2.07 (2H, quint, $J$ = 7.2 Hz), 2.33 (2H, t, $J$ = 7.2 Hz), 2.42 (2H, q, $J$ = 7.2 Hz), 2.80−2.85 (10H, m), 4.07 (2H, d, $J$ = 7.2 Hz), 5.27−5.41 (12H, m), 6.23 (1H, s). $^{13}$C NMR: $\delta$ ($CDCl_3$) 14.26, 20.53, 23.13, 25.50, 25.59, 35.93, 41.45, 126.97, 127.68, 127.83, 127.91, 128.03, 128.25, 128.33, 128.55, 129.68, 132.03, 172.88, 173.59. HRMS (ESI) $m/z$: calcd for $C_{24}H_{35}NO_3$ [M + H]$^+$, 386.2695; found [M + H]$^+$, 386.2722.

*Methyl 2-((4Z,7Z,10Z,13Z,16Z,19Z)-Docosa-4,7,10,13,16,19-hexaenamido)acetate (**33**).* A mixture of 2-aminoacetic acid (0.50 g, 6.66 mmol) and chlorotrimethylsilane (1.45 g, 13.3 mmol) was stirred at room temperature for 10 min. Methanol (6 mL) was then added to the mixture, and the reaction was stirred for 48 h at room temperature. The produced solid was filtered, washed with ethyl acetate, and dried under the vacuum to afford methyl 2-aminoacetate hydrochloride in 100% yield.

To a solution of DHA (0.10 g, 0.30 mmol), DMAP (37 mg, 0.30 mmol), triethylamine (31 mg, 0.30 mmol) in dry dichloromethane (5 mL) was added methyl 2-aminoacetate hydrochloride (38 mg, 0.30 mmol) at room temperature. After stirring for 5 min, EDCI (58 mg, 0.30 mmol) was added to the mixture, and the reaction was stirred overnight at room temperature under nitrogen gas. The product was extracted with ethyl acetate (50 mL × 2). The combined organic solutions were washed with an aqueous solution of 1 N hydrochloric acid (15 mL) and brine (70 mL × 2), dried over magnesium sulfate, and evaporated to dryness. The residue was purified by using silica gel column chromatography (hexane/ethyl acetate = 5:1) to afford compound **33** in 97% yield. $^1$H NMR: $\delta$ ($CDCl_3$) 0.97 (3H, t, $J$ = 7.2 Hz), 2.07 (2H, quint, $J$ = 7.2 Hz), 2.30 (2H, t, $J$ = 7.2 Hz), 2.41 (2H, q, $J$ = 7.2 Hz), 2.80−2.85 (10H, m), 3.76 (3H, s), 4.05 (2H, d, $J$ = 7.2 Hz),


DOI: 10.1021/acs.jmedchem.9b00994
J. Med. Chem. 2019, 62, 9576−9592

5.27−5.41 (12H, m), 5.97 (1H, s). $^{13}$C NMR: δ ($CDCl_3$) 14.27, 20.54, 23.19, 25.51, 25.61, 36.05, 41.19, 52.39, 126.99, 127.85, 128.02, 128.06, 128.24, 128.54, 129.45, 132.02, 170.49, 172.42. HRMS (ESI) *m/z*: calcd for $C_{25}H_{37}NO_3$ [M + H]$^+$, 400.2852; found [M + H]$^+$, 400.2875.

*(4Z,7Z,10Z,13Z,16Z,19Z)-N-(3-Hydroxypropyl)docosa-4,7,10,13,16,19-hexaenamide (**34**).* $^1$H NMR: δ ($CDCl_3$) 0.97 (3H, t, *J* = 7.2 Hz), 1.64−1.69 (2H, m), 2.07 (2H, quint, *J* = 7.2 Hz), 2.26 (2H, t, *J* = 7.2 Hz), 2.41 (2H, q, *J* = 7.2 Hz), 2.80−2.86 (10H, m), 3.30 (1H, s), 3.41 (2H, q, *J* = 7.2 Hz), 3.61 (2H, t, *J* = 7.2 Hz), 5.27−5.41 (12H, m), 5.85 (1H, s). $^{13}$C NMR: δ ($CDCl_3$) 14.26, 20.53, 23.40, 25.50, 25.60, 32.23, 36.06, 36.37, 59.01, 129.96, 127.82, 127.95, 128.00, 128.03, 128.24, 128.30, 128.55, 129.51, 132.03, 173.71. HRMS (ESI) *m/z*: calcd for $C_{25}H_{39}NO_2$ [M + H]$^+$, 386.3059; found [M + H]$^+$, 386.3098.

*(4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-(2-Hydroxyethoxy)ethyl)docosa-4,7,10,13,16,19-hexaenamide (**35**).* $^1$H NMR: δ ($CDCl_3$) 0.97 (3H, t, *J* = 7.2 Hz), 1.73 (1H, s), 2.07 (2H, quint, *J* = 7.2 Hz), 2.24 (2H, t, *J* = 7.2 Hz), 2.42 (2H, q, *J* = 7.2 Hz), 2.80−2.85 (10H, m), 3.45 (2H, q, *J* = 7.2 Hz), 3.56 (4H, m), 3.73 (2H, t, *J* = 7.2 Hz), 5.26−5.42 (12H, m), 5.97 (1H, s). $^{13}$C NMR: δ ($CDCl_3$) 14.26, 20.53, 23.35, 25.50, 25.59, 36.39, 39.17, 61.74, 69.93, 72.13, 126.97, 127.83, 128.04, 128.20, 128.25, 128.54, 129.26, 132.02, 172.51. HRMS (ESI) *m/z*: calcd for $C_{26}H_{41}NO_3$ [M + H]$^+$, 416.3165; found [M + H]$^+$, 416.3207.

*(4Z,7Z,10Z,13Z,16Z,19Z)-2-(2-Hydroxyethoxy)ethyl Docosa-4,7,10,13,16,19-hexaenoate (**36**).* $^1$H NMR: δ ($CDCl_3$) 0.97 (3H, t, *J* = 7.2 Hz), 2.07 (2H, quint, *J* = 7.2 Hz), 2.38−2.43 (4H, m), 2.80−2.88 (10H, m), 3.60 (2H, t, *J* = 7.2 Hz), 3.69−3.77 (4H, m), 4.25 (2H, t, *J* = 7.2 Hz), 5.25−5.44 (12H, m). $^{13}$C NMR: δ ($CDCl_3$) 14.26, 20.53, 22.68, 22.50, 25.55, 25.59, 34.03, 61.70, 63.36, 69.10, 72.27, 126.98, 127.78, 127.83, 128.02, 128.08, 128.20, 128.23, 128.53, 129.32, 132.01, 173.08. HRMS (ESI) *m/z*: calcd for $C_{26}H_{40}O_4$ [M + H]$^+$, 417.3005; found [M + H]$^+$, 417.3022.

*(4Z,7Z,10Z,13Z,16Z,19Z)-N-(2-Hydroxyphenyl)docosa-4,7,10,13,16,19-hexaenamide (**37**).* $^1$H NMR: δ ($CDCl_3$) 0.97 (3H, t, *J* = 7.2 Hz), 2.07 (2H, quint, *J* = 7.2 Hz), 2.49−2.53 (4H, m), 2.77−2.88 (10H, m), 5.27−5.54 (12H, m), 6.83−6.87 (1H, m), 6.94−6.96 (1H, m), 7.00−7.03 (1H, m), 7.11−7.15 (1H, m), 7.48 (1H, s), 8.76 (1H, s). $^{13}$C NMR: δ ($CDCl_3$) 14.27, 20.53, 23.36, 25.51, 25.61, 36.64, 120.02, 120.37, 122.06, 125.40, 126.97, 127.27, 127.67, 127.83, 127.96, 128.01, 128.27, 128.50, 128.55, 130.41, 132.04, 148.80, 172.73. HRMS (ESI) *m/z*: calcd for $C_{28}H_{37}NO_2$ [M + H]$^+$, 420.2903; found [M + H]$^+$, 420.2917.

*(4Z,7Z,10Z,13Z,16Z,19Z)-N-(3-Hydroxyphenyl)docosa-4,7,10,13,16,19-hexaenamide (**38**).* $^1$H NMR: δ ($CDCl_3$) 0.97 (3H, t, *J* = 7.2 Hz), 2.07 (2H, quint, *J* = 7.2 Hz), 2.47−2.53 (2H, m), 2.57−2.61 (2H, m), 2.79−2.91 (10H, m), 3.71 (2H, s), 5.27−5.49 (12H, m), 6.39 (1H, t, *J* = 2.4 Hz), 6.45 (1H, d, *J* = 8.8 Hz), 6.52 (1H, d, *J* = 8.8 Hz), 7.13 (1H, t, *J* = 8.0 Hz). $^{13}$C NMR: δ ($CDCl_3$) 14.28, 20.54, 22.80, 25.51, 25.60, 34.29, 108.22, 111.36, 112.55, 127.58, 128.01, 128.05, 128.22, 128.28, 129.59, 130.04, 132.04, 147.58, 151.65, 171.57. HRMS (ESI) *m/z*: calcd for $C_{28}H_{37}NO_2$ [M + H]$^+$, 420.2903; found [M + H]$^+$, 420.2939.

*(4Z,7Z,10Z,13Z,16Z,19Z)-N-(3,4-Dihydroxyphenethyl)docosa-4,7,10,13,16,19-hexaenamide (**39**).* $^1$H NMR: δ ($CDCl_3$) 0.96 (3H, t, *J* = 7.2 Hz), 2.07 (2H, quint, *J* = 7.2 Hz), 2.21 (2H, t, *J* = 7.2 Hz), 2.38 (2H, q, *J* = 7.2 Hz), 2.68 (2H, t, *J* = 7.2 Hz), 2.78−2.87 (10H, m), 3.47 (2H, q, *J* = 7.2 Hz), 5.27−5.43 (12H, m), 5.68 (1H, s), 6.07 (1H, s), 6.56 (1H, d, *J* = 7.2 Hz), 6.75 (1H, s), 6.80 (1H, d, *J* = 7.2 Hz), 7.74 (1H, s). $^{13}$C NMR: δ ($CDCl_3$) 14.27, 20.54, 23.34, 25.56, 25.61, 34.92, 36.49, 41.09, 115.07, 115.35, 120.41, 126.99, 127.69, 127.84, 128.04, 128.29, 128.38, 128.58, 129.71, 132.01, 143.11, 144.27, 173.54. HRMS (ESI) *m/z*: calcd for $C_{30}H_{41}NO_3$ [M + H]$^+$, 464.3165; found [M + H]$^+$, 464.3162.

*(4Z,7Z,10Z,13Z,16Z,19Z)-1-Morpholinodocosa-4,7,10,13,16,19-hexaen-1-one (**40**).* $^1$H NMR: δ ($CDCl_3$) 0.97 (3H, t, *J* = 7.2 Hz), 2.07 (2H, quint, *J* = 7.2 Hz), 2.34−2.45 (4H, m), 2.80−2.88 (10H, m), 3.46 (2H, t, *J* = 7.2 Hz), 3.61−3.68 (6H, m), 5.27−5.44 (12H, m). $^{13}$C NMR: δ ($CDCl_3$) 14.27, 20.53, 22.93, 25.51, 25.59, 25.60, 32.82, 41.86, 45.89, 66.60, 66.91, 126.97, 127.83, 128.03, 128.07, 128.23, 128.40, 128.54, 129.05, 132.01, 170.98. HRMS (ESI) *m/z*: calcd for $C_{26}H_{39}NO_2$ [M + H]$^+$, 398.3059; found [M + H]$^+$, 398.3102.

*(4Z,7Z,10Z,13Z,16Z,19Z)-N-(Tetrahydro-2H-pyran-4-yl)docosa-4,7,10,13,16,19-hexaenamide (**41**).* $^1$H NMR: δ ($CDCl_3$) 0.97 (3H, t, *J* = 7.2 Hz), 1.38−1.48 (2H, m), 1.86−1.91 (2H, m), 2.07 (2H, quint, *J* = 7.2 Hz), 2.21 (2H, t, *J* = 7.2 Hz), 2.41 (2H, q, *J* = 7.2 Hz), 2.80−2.88 (10H, m), 3.47 (2H, t, *J* = 7.2 Hz), 3.92−4.05 (3H, m), 5.27−5.44 (13H, m). $^{13}$C NMR: δ ($CDCl_3$) 14.28, 20.54, 23.39, 25.52, 25.60, 33.20, 36.52, 45.57, 66.77, 126.97, 127.83, 127.99, 128.03, 128.15, 128.25, 128.56, 129.37, 132.03, 171.52. HRMS (ESI) *m/z*: calcd for $C_{27}H_{41}NO_2$ [M + H]$^+$, 412.3216; found [M + H]$^+$, 412.3252.

*(4Z,7Z,10Z,13Z,16Z,19Z)-N-((Tetrahydro-2H-pyran-4-yl)methyl)-docosa-4,7,10,13,16,19-hexaenamide (**42**).* $^1$H NMR: δ ($CDCl_3$) 0.97 (3H, t, *J* = 7.2 Hz), 1.24−1.35 (2H, m), 1.57−1.61 (2H, m), 1.69−1.80 (1H, m), 2.07 (2H, quint, *J* = 7.2 Hz), 2.23 (2H, t, *J* = 7.2 Hz), 2.41 (2H, q, *J* = 7.2 Hz), 2.80−2.88 (10H, m), 3.15 (2H, t, *J* = 7.2 Hz), 3.35 (2H, t, *J* = 7.2 Hz), 3.96 (2H, d, *J* = 7.2 Hz), 5.27−5.44 (12H, m), 5.53 (1H, s). $^{13}$C NMR: δ ($CDCl_3$) 14.27, 20.53, 23.41, 25.51, 25.60, 30.57, 35.22, 36.50, 45.13, 67.56, 126.96, 127.81, 127.97, 128.02, 128.21, 128.26, 128.55, 129.38, 132.03, 172.38. HRMS (ESI) *m/z*: calcd for $C_{28}H_{43}NO_2$ [M + H]$^+$, 426.3372; found [M + H]$^+$, 426.3406.

*(4Z,7Z,10Z,13Z,16Z,19Z)-1-(4-Methylpeperazin-1-yl)docosa-4,7,10,13,16,19-hexaen-1-one (**43**).* $^1$H NMR: δ ($CDCl_3$) 0.97 (3H, t, *J* = 7.2 Hz), 2.07 (2H, quint, *J* = 7.2 Hz), 2.30 (3H, s), 2.34−2.47 (8H, m), 2.80−2.88 (10H, m), 3.47 (2H, t, *J* = 7.2 Hz), 3.64 (2H, t, *J* = 7.2 Hz), 5.27−5.44 (12H, m). $^{13}$C NMR: δ ($CDCl_3$) 14.27, 20.53, 23.05, 25.51, 25.59, 33.00, 41.45, 45.39, 46.04, 54.70, 55.13, 126.98, 127.84, 128.05, 128.09, 128.15, 128.20, 128.23, 128.53, 128.56, 128.90, 132.01, 170.75. HRMS (ESI) *m/z*: calcd for $C_{27}H_{42}N_2O$ [M + H]$^+$, 411.3375; found [M + H]$^+$, 411.3403.

*(4Z,7Z,10Z,13Z,16Z,19Z)-1-(4-(Methylsulfonyl)peperazin-1-yl)-docosa-4,7,10,13,16,19-hexaen-1-one (**44**).* $^1$H NMR: δ ($CDCl_3$) 0.97 (3H, t, *J* = 7.2 Hz), 2.07 (2H, quint, *J* = 7.2 Hz), 2.36−2.44 (4H, m), 2.79 (3H, s), 2.81−2.88 (10H, m), 3.19−3.24 (4H, m), 3.57−3.59 (2H, m), 3.74−3.76 (2H, m), 5.28−5.45 (12H, m). $^{13}$C NMR: δ ($CDCl_3$) 14.27, 20.52, 22.85, 25.50, 25.58, 32.95, 34.70, 41.08, 45.10, 45.62, 45.87, 126.95, 127.81, 128.00, 128.16, 128.26, 128.30, 128.55, 129.24, 132.02, 170.85. HRMS (ESI) *m/z*: calcd for $C_{27}H_{42}N_2O_3S$ [M + H]$^+$, 475.2994; found [M + H]$^+$, 475.2996.

*(4Z,7Z,10Z,13Z,16Z,19Z)-N-(Methylsulfonyl)docosa-4,7,10,13,16,19-hexaenamide (**45**).* $^1$H NMR: δ ($CDCl_3$) 0.97 (3H, t, *J* = 7.2 Hz), 2.07 (2H, quint, *J* = 7.2 Hz), 2.37−2.47 (4H, m), 2.80−2.88 (10H, m), 3.30 (3H, s), 5.27−5.41 (12H, m), 8.22 (1H, s). $^{13}$C NMR: δ ($CDCl_3$) 14.27, 20.53, 22.13, 25.51, 25.61, 36.26, 41.60, 126.82, 126.97, 127.61, 127.82, 127.94, 128.01, 128.29, 128.32, 128.56, 130.48, 132.04, 171.06. HRMS (ESI) *m/z*: calcd for $C_{23}H_{35}NO_3S$ [M + H]$^+$, 406.2416; found [M + H]$^+$, 406.2459.

*(4Z,7Z,10Z,13Z,16Z,19Z)-N-(tert-Butylsulfonyl)docosa-4,7,10,13,16,19-hexaenamide (**46**).* $^1$H NMR: δ ($CDCl_3$) 0.97 (3H, t, *J* = 7.2 Hz), 1.47 (9H, s), 2.07 (2H, quint, *J* = 7.2 Hz), 2.43−2.50 (4H, m), 2.80−2.86 (10H, m), 5.28−5.41 (12H, m), 7.82 (1H, s). $^{13}$C NMR: δ ($CDCl_3$) 14.28, 20.54, 22.36, 24.31, 25.52, 25.61, 36.44, 62.11, 126.98, 127.13, 127.77, 127.84, 128.03, 128.27, 128.44, 128.56, 130.19, 132.03, 170.75. HRMS (ESI) *m/z*: calcd for $C_{26}H_{41}NO_3S$ [M + H]$^+$, 448.2885; found [M + H]$^+$, 448.2920.

*(4Z,7Z,10Z,13Z,16Z,19Z)-N-(tert-Butyl)docosa-4,7,10,13,16,19-hexaenamide (**47**).* $^1$H NMR: δ ($CDCl_3$) 0.97 (3H, t, *J* = 7.2 Hz), 1.34 (9H, s), 2.07 (2H, quint, *J* = 7.2 Hz), 2.14 (2H, t, *J* = 7.2 Hz), 2.38 (2H, q, *J* = 7.2 Hz), 2.80−2.86 (10H, m), 5.28−5.41 (13H, m). $^{13}$C NMR: δ ($CDCl_3$) 14.27, 20.54, 23.47, 25.51, 25.59, 28.80, 37.34, 51.14, 126.99, 127.85, 128.06, 128.11, 128.21, 128.24, 128.39, 128.54, 129.09, 132.02, 171.65. HRMS (ESI) *m/z*: calcd for $C_{26}H_{41}NO$ [M + H]$^+$, 384.3266; found [M + H]$^+$, 384.3279.

*(4Z,7Z,10Z,13Z,16Z,19Z)-N-(Cyclopropylsulfonyl)docosa-4,7,10,13,16,19-hexaenamide (**48**).* $^1$H NMR: δ ($CDCl_3$) 0.97 (3H, t, *J* = 7.2 Hz), 1.12 (2H, d, *J* = 7.2 Hz), 1.38 (2H, d, *J* = 7.2 Hz), 2.07 (2H, quint, *J* = 7.2 Hz), 2.37−2.47 (4H, m), 2.74−2.88 (10H, m), 2.90−2.97 (1H, m), 5.28−5.50 (12H, m), 8.06 (1H, s). $^{13}$C NMR: δ ($CDCl_3$) 6.31, 14.26, 20.53, 22.23, 25.52, 25.62, 31.32, 36.21, 126.97, 127.68, 127.83, 127,97, 128.02, 128,29, 128,32, 128.52, 128.57, 130.36, 132.04,


DOI: 10.1021/acs.jmedchem.9b00994
J. Med. Chem. 2019, 62, 9576−9592

170.63. HRMS (ESI) $m/z$: calcd for $C_{25}H_{37}NO_3S$ [M + H]$^+$, 432.2572; found [M + H]$^+$, 432.2579.

*(4Z,7Z,10Z,13Z,16Z,19Z)-N-(Trifluoromethylsulfonyl)docosa-4,7,10,13,16,19-hexaenamide (**49**).* $^1$H NMR: $\delta$ ($CDCl_3$) 0.97 (3H, t, $J$ = 7.2 Hz), 2.07 (2H, quint, $J$ = 7.2 Hz), 2.45 (2H, q, $J$ = 7.2 Hz), 2.58 (2H, t, $J$ = 7.2 Hz), 2.80−2.88 (10H, m), 5.27−5.53 (12H, m). $^{13}$C NMR: $\delta$ ($CDCl_3$) 14.25, 20.54, 21.84, 25.52, 25.57, 25.61, 36.01, 126.41, 126.99, 127.52, 127.84, 127.94, 128.02, 128.34, 128.62, 130.84, 132.07, 169.57. HRMS (ESI) $m/z$: calcd for $C_{23}H_{32}F_3NO_3S$ [M − H]$^-$, 458.1977; found [M − H]$^-$, 458.1966.

*(4Z,7Z,10Z,13Z,16Z,19Z)-N-(Pentafluoroethanesulfonyl)docosa-4,7,10,13,16,19-hexaenamide (**50**).* $^1$H NMR: $\delta$ ($CDCl_3$) 0.96 (3H, t, $J$ = 7.2 Hz), 2.06 (2H, quint, $J$ = 7.2 Hz), 2.32 (4H, s), 2.78−2.84 (10H, m), 3.18 (1H, s), 5.26−5.42 (12H, m). $^{13}$C NMR: $\delta$ ($CDCl_3$) 14.23, 20.52, 22.92, 25.40, 25.49, 25.54, 30.96, 39.69, 126.97, 127.81, 128.02, 128.04, 128.18, 128.25, 128.53, 128.88, 131.99, 183.77. HRMS (ESI) $m/z$: calcd for $C_{24}H_{32}F_5NO_3S$ [M − H]$^-$, 508.1945; found [M − H]$^-$, 508.1927.

*(4Z,7Z,10Z,13Z,16Z,19Z)-N-(Phenylsulfonyl)docosa-4,7,10,13,16,19-hexaenamide (**51**).* $^1$H NMR: $\delta$ ($CDCl_3$) 0.96 (3H, t, $J$ = 7.2 Hz), 2.07 (2H, quint, $J$ = 7.2 Hz), 2.29−2.37 (4H, m), 2.72−2.84 (10H, m), 5.22−5.42 (12H, m), 7.55 (2H, t, $J$ = 8.0 Hz), 7.66 (1H, t, $J$ = 8.0 Hz), 8.06 (2H, d, $J$ = 8.0 Hz), 8.31 (1H, s). $^{13}$C NMR: $\delta$ ($CDCl_3$) 14.26, 20.53, 22.05, 25.51, 25.59, 36.09, 126.85, 126.98, 127.66, 127.84, 127.97, 128.02, 128.28, 128.34, 128.45, 128.57, 129.00, 130.28, 132.04, 134.03, 169.88. HRMS (ESI) $m/z$: calcd for $C_{28}H_{37}NO_3S$ [M + H]$^+$, 468.2572; found [M + H]$^+$, 468.2580.

*(4Z,7Z,10Z,13Z,16Z,19Z)-N-(Benzylsulfonyl)docosa-4,7,10,13,16,19-hexaenamide (**52**).* $^1$H NMR: $\delta$ ($CDCl_3$) 0.97 (3H, t, $J$ = 7.2 Hz), 2.07 (2H, quint, $J$ = 7.2 Hz), 2.27 (2H, t, $J$ = 7.2 Hz), 2.38 (2H, q, $J$ = 7.2 Hz), 2.79−2.88 (10H, m), 4.63 (2H, s), 5.27−5.49 (12H, m), 7.32−7.42 (5H, m), 7.63 (1H, s). $^{13}$C NMR: $\delta$ ($CDCl_3$) 14.27, 20.54, 22.06, 25.53, 25.58, 25.63, 36.13, 58.83, 126.92, 126.99, 127.61, 127.84, 127.96, 128.02, 128.32, 128.36, 128.59, 128.98, 130.48, 130.66, 132.06, 171.13. HRMS (ESI) $m/z$: calcd for $C_{29}H_{39}NO_3S$ [M + H]$^+$, 482.2729; found [M + H]$^+$, 482.2730.

*(4Z,7Z,10Z,13Z,16Z,19Z)-N-Tosyldocosa-4,7,10,13,16,19-hexaenamide (**53**).* $^1$H NMR: $\delta$ ($CDCl_3$) 0.96 (3H, t, $J$ = 7.2 Hz), 2.07 (2H, quint, $J$ = 7.2 Hz), 2.28−2.34 (4H, m), 2.44 (3H, s), 2.73−2.84 (10H, m), 5.23−5.41 (12H, m), 7.34 (2H, d, $J$ = 8.4 Hz), 7.93 (2H, d, $J$ = 8.4 Hz), 8.30 (1H, s). $^{13}$C NMR: $\delta$ ($CDCl_3$) 14.26, 20.53, 21.69, 22.06, 25.49, 25.59, 36.07, 126.92, 126.98, 127.69, 127.84, 127.98, 128.02, 128.28, 128.40, 128.56, 129.61, 130.22, 132.04, 145.17, 169.91. HRMS (ESI) $m/z$: calcd for $C_{29}H_{39}NO_3S$ [M + H]$^+$, 482.2729; found [M + H]$^+$, 482.2720.

*(4Z,7Z,10Z,13Z,16Z,19Z)-N-(4-Hydroxyphenylsulfonyl)docosa-4,7,10,13,16,19-hexaenamide (**54**).* $^1$H NMR: $\delta$ ($CDCl_3$) 0.97 (3H, t, $J$ = 7.2 Hz), 2.07 (2H, quint, $J$ = 7.2 Hz), 2.52 (2H, q, $J$ = 7.2 Hz), 2.66 (2H, t, $J$ = 7.2 Hz), 2.79−2.91 (10H, m), 4.84 (2H, s), 5.27−5.52 (12H, m), 7.23−7.26 (2H, m), 7.94−7.96 (2H, m). $^{13}$C NMR: $\delta$ ($CDCl_3$) 14.27, 20.54, 22.62, 25.52, 25.62, 34.20, 122.44, 126.98, 127.14, 127.82, 128.02, 128.20, 128.28, 128.43, 128.58, 130.00, 132.06, 139.13, 153.91, 170.94. HRMS (ESI) $m/z$: calcd for $C_{28}H_{37}NO_4S$ [M − H]$^-$, 482.2365; found [M − H]$^-$, 482.2407.

*(4Z,7Z,10Z,13Z,16Z,19Z)-N-(4-Fluorophenylsulfonyl)docosa-4,7,10,13,16,19-hexaenamide (**55**).* $^1$H NMR: $\delta$ ($CDCl_3$) 0.96 (3H, t, $J$ = 7.2 Hz), 2.07 (2H, quint, $J$ = 7.2 Hz), 2.28−2.36 (4H, m), 2.74−2.88 (10H, m), 5.23−5.43 (12H, m), 7.19−7.26 (2H, m), 8.07−8.12 (2H, m). $^{13}$C NMR: $\delta$ ($CDCl_3$) 14.27, 20.53, 22.02, 25.50, 25.59, 36.12, 116.18, 116.41, 126.79, 126.98, 127.58, 127.81, 127.92, 128.01, 128.28, 128.50, 128.56, 130.35, 131.36, 131.47, 132.04, 134.35, 164.64, 169.87. HRMS (ESI) $m/z$: calcd for $C_{28}H_{36}FNO_3S$ [M + H]$^+$, 486.2478; found [M + H]$^+$, 486.2518.

*(4Z,7Z,10Z,13Z,16Z,19Z)-N-(4-Chlorophenylsulfonyl)docosa-4,7,10,13,16,19-hexaenamide (**56**).* $^1$H NMR: $\delta$ ($CDCl_3$) 0.97 (3H, t, $J$ = 7.2 Hz), 2.07 (2H, quint, $J$ = 7.2 Hz), 2.28−2.36 (4H, m), 2.74−2.85 (10H, m), 5.23−5.44 (12H, m), 7.52 (2H, d, $J$ = 8.4 Hz), 8.00 (2H, d, $J$ = 8.4 Hz), 8.25 (1H, s). $^{13}$C NMR: $\delta$ ($CDCl_3$) 14.27, 20.54, 22.04, 25.52, 25.61, 36.16, 126.75, 126.99, 127.58, 127.84, 127.95, 128.02, 128.33, 128.54, 128.59, 129.31, 129.94, 130.47, 132.06, 136.82, 169.79. HRMS (ESI) $m/z$: calcd for $C_{28}H_{36}ClNO_3S$ [M + H]$^+$, 502.2183; found [M + H]$^+$, 502.2251.

*(4Z,7Z,10Z,13Z,16Z,19Z)-N-(4-(Trifluoromethyl)phenylsulfonyl)docosa-4,7,10,13,16,19-hexaenamide (**57**).* $^1$H NMR: $\delta$ ($CDCl_3$) 0.96 (3H, t, $J$ = 7.2 Hz), 2.07 (2H, quint, $J$ = 7.2 Hz), 2.30−2.38 (4H, m), 2.74−2.88 (10H, m), 5.23−5.44 (12H, m), 7.81 (2H, d, $J$ = 8.0 Hz), 8.20 (2H, d, $J$ = 8.0 Hz), 8.35 (1H, s). $^{13}$C NMR: $\delta$ ($CDCl_3$) 14.23, 20.53, 22.00, 25.52, 25.60, 36.18, 126.14, 126.17, 126.66, 126.98, 127.51, 127.83, 127.91, 128.00, 128.31, 128.34, 128.58, 129.06, 130.56, 132.04, 141.83, 169.86. HRMS (ESI) $m/z$: calcd for $C_{29}H_{36}F_3NO_3S$ [M + H]$^+$, 536.2446; found [M + H]$^+$, 536.2483.

*(4Z,7Z,10Z,13Z,16Z,19Z)-N-(4-Trifluoromethoxyphenylsulfonyl)docosa-4,7,10,13,16,19-hexaenamide (**58**).* $^1$H NMR: $\delta$ ($CDCl_3$) 0.96 (3H, t, $J$ = 7.2 Hz), 2.07 (2H, quint, $J$ = 7.2 Hz), 2.24−2.37 (4H, m), 2.75−2.85 (10H, m), 5.23−5.44 (12H, m), 7.36 (2H, d, $J$ = 8.4 Hz), 8.13 (2H, d, $J$ = 8.4 Hz), 8.25 (1H, s). NMR: $\delta$ ($CDCl_3$) 14.25, 20.53, 22.02, 25.52, 25.60, 36.17, 120.58, 126.72, 126.98, 127.55, 127.83, 127.92, 128.01, 128.34, 128.56, 130.50, 130.83, 132.05, 136.49, 153.14, 169.80. HRMS (ESI) $m/z$: calcd for $C_{29}H_{36}F_3NO_4S$ [M + H]$^+$, 552.2395; found [M + H]$^+$, 552.2465.

*(4Z,7Z,10Z,13Z,16Z,19Z)-N-(4-Nitrophenylsulfonyl)docosa-4,7,10,13,16,19-hexaenamide (**59**).* $^1$H NMR: $\delta$ ($CDCl_3$) 0.97 (3H, t, $J$ = 7.2 Hz), 2.07 (2H, quint, $J$ = 7.2 Hz), 2.30−2.37 (4H, m), 2.75−2.87 (10H, m), 5.23−5.46 (12H, m), 8.28 (2H, d, $J$ = 8.4 Hz), 8.38 (2H, d, $J$ = 8.4 Hz). $^{13}$C NMR: $\delta$ ($CDCl_3$) 14.27, 20.54, 21.98, 25.53, 25.61, 36.21, 124.15, 126.57, 126.98, 127.44, 127.82, 127.98, 128.34, 128.39, 128.61, 129.99, 130.75, 132.07, 143.85, 150.83, 169.72. HRMS (ESI) $m/z$: calcd for $C_{28}H_{36}N_2O_5S$ [M − H]$^-$, 511.2267; found [M − H]$^-$, 511.2245.

*(4Z,7Z,10Z,13Z,16Z,19Z)-N-(4-Carboxyphenylsulfonyl)docosa-4,7,10,13,16,19-hexaenamide (**60**).* $^1$H NMR: $\delta$ ($CDCl_3$) 0.96 (3H, t, $J$ = 7.2 Hz), 2.07 (2H, quint, $J$ = 7.2 Hz), 2.32−2.38 (4H, m), 2.75−2.85 (10H, m), 5.24−5.45 (12H, m), 8.17 (2H, d, $J$ = 8.4 Hz), 8.26 (2H, d, $J$ = 8.4 Hz), 8.40 (1H, s). $^{13}$C NMR: $\delta$ ($CDCl_3$) 14.25, 20.53, 22.01, 25.51, 25.59, 36.15, 126.71, 126.98, 127.57, 127.83, 127.92, 128.01, 128.30, 128.54, 128.58, 130.72, 132.05, 134.02, 142.97, 169.18, 170.10. HRMS (ESI) $m/z$: calcd for $C_{29}H_{37}NO_5S$ [M + H]$^+$, 512.2471; found [M + H]$^+$, 512.2474.

*(4Z,7Z,10Z,13Z,16Z,19Z)-N-((4-Methoxycarbonyl)-phenylsulfonyl)docosa-4,7,10,13,16,19-hexaenamide (**61**).* $^1$H NMR: $\delta$ ($CDCl_3$) 0.97 (3H, t, $J$ = 7.2 Hz), 2.07 (2H, quint, $J$ = 7.2 Hz), 2.29−2.36 (4H, m), 2.73−2.87 (10H, m), 3.96 (3H, s), 5.22−5.44 (12H, m), 8.13 (2H, d, $J$ = 8.4 Hz), 8.20 (2H, d, $J$ = 8.4 Hz), 8.26 (1H, s). $^{13}$C NMR: $\delta$ ($CDCl_3$) 14.26, 20.54, 22.02, 25.51, 25.60, 36.16, 52.75, 126.72, 126.99, 127.57, 127.84, 127.93, 128.30, 128.32, 128.48, 128.53, 128.58, 130.13, 130.49, 132.05, 134.02, 142.18, 165.41, 169.78. HRMS (ESI) $m/z$: calcd for $C_{30}H_{39}NO_5S$ [M + H]$^+$, 526.2627; found [M + H]$^+$, 526.2635.

*(4Z,7Z,10Z,13Z,16Z,19Z)-N-((2-Thiophene)sulfonyl)docosa-4,7,10,13,16,19-hexaenamide (**62**).* $^1$H NMR: $\delta$ ($CDCl_3$) 0.97 (3H, t, $J$ = 7.2 Hz), 2.07 (2H, quint, $J$ = 7.2 Hz), 2.33−2.41 (4H, m), 2.76−2.88 (10H, m), 5.26−5.45 (12H, m), 7.12 (1H, t, $J$ = 8.0 Hz), 7.70 (1H, d, $J$ = 8.0 Hz), 7.90 (1H, d, $J$ = 8.0 Hz), 8.32 (1H, s). $^{13}$C NMR: $\delta$ ($CDCl_3$) 14.26, 20.54, 22.08, 25.52, 25.60, 36.15, 126.84, 126.98, 127.40, 127.65, 127.84, 127.98, 128.02, 128.29, 128.48, 128.57, 130.39, 132.05, 134.07, 135.30, 169.87. HRMS (ESI) $m/z$: calcd for $C_{26}H_{35}NO_3S_2$ [M + H]$^+$, 474.2137; found [M + H]$^+$, 474.2147.

**β-Hexosaminidase Release Assay.** The assay was performed as previously reported with some modifications.[41] RBL-2H3 cells (American Type Culture Collection, Rockvile, MD, USA) were cultured in DMEM supplemented with 100 U/mL of penicillin, 100 μg/mL of streptomycin, and 10% heat-inactivated FBS at 37 °C under humidified 5% $CO_2$ in air. RBL-2H3 cells were seeded at 4.0 × 10$^4$ cells/well in a 96-well culture plate and sensitized with anti-DNP IgE (50 ng/mL) at 37 °C overnight. After washing the cells with modified Tyrode's (MT) buffer (20 mM HEPES, 135 mM NaCl, 5 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, 5.6 mM glucose, and 0.05% BSA, pH 7.4) twice, the cells were treated with 120 μL of MT buffer containing various concentrations of compounds or 0.1% (v/v) ethanol (vehicle) for 30 min at 37 °C. When the effect of CB2 antagonism on DHEA


DOI: 10.1021/acs.jmedchem.9b00994
J. Med. Chem. 2019, 62, 9576−9592

activity was investigated, the cells were treated with various concentrations of DHEA in the presence of 10 μM AM630 (a CB2 antagonist) for 30 min. When the effect of FAAH inhibition on DHEA activity was investigated, the cells were treated with various concentrations of DHEA in the presence of 10 μM URB597 (a FAAH inhibitor) for 30 min. The cells were then stimulated with 10 μL of DNP−human serum albumin (HSA) (625 ng/mL) for 30 min at 37 °C. After stimulation, the supernatant was collected from each well, and the cells were sonicated in 130 μL of MT buffer containing 0.1% Triton X-100 for 5 s on ice. Both the supernatant and cell lysate were transferred to a new 96-well microplate at 50 μL/well and incubated for 5 min at 37 °C. One hundred μL of 3.3 mM 4-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside dissolved in 0.1 M citrate buffer (pH 4.5) was then added to each well and incubated for 25 min at 37 °C. The enzyme reaction was terminated by adding 100 μL of 2 M glycine buffer (pH 10.4), and the absorbance was measured at 405 nm using a microplate reader (SH-8000Lab; Corona Electric, Hitachinaka, Japan). A β-hexosaminidase release rate (%) was calculated as follows

$$\left[\frac{(A_{\text{supernatant}}-A_{\text{blank of supernatant}})}{\{(A_{\text{supernatant}}-A_{\text{blank of supernatant}})+(A_{\text{cell lysate}}-A_{\text{blank of cell lysate}})\}}\right]$$

in which "A" is the absorbance of each well.

**IgE-Mediated PCA in Mice.** An IgE-dependent PCA experiment was conducted according to the method of Nishi et al. with some modifications.[31] Female BALB/c mice were purchased from Clea Japan (Tokyo, Japan) and housed in a temperature-controlled (24 ± 2 °C) environment under 12 h light/dark cycle. Animals received standard chow (Rodent LabDiet EQ 5L37; Nutrition International, Brentwood, MO, USA) and water ad libitum. After acclimating to their housing environment for 1 week, 8-week-old female BALB/c mice were randomly divided into groups (five mice per group). Mice were orally administered 20 μL of test compounds (DHEA, **26**, **32**, **34**, **35**, **46**, **52**, **55**, and **57**) or 20 μL of water as a control. In the case of solid compounds (**28**, **29**, **58**, and **59**), mice were orally administered 20 mg of test compounds suspended in 10 μL of mineral oil or 30 μL of mineral oil as a control. Oral administration was conducted once a day for 5 consecutive days (day 0 to day 4). On day 3, all mice were intradermally injected with 0.1 μg of anti-DNP IgE dissolved in 10 μL of PBS and with 10 μL of PBS alone into their left and right ears, respectively. On day 4, 0.2 mg of DNP−HSA and 0.5% (w/v) Evans blue dye dissolved in 0.2 mL of PBS were intravenously injected 1 h after oral administration. The mice were euthanized 30 min after intravenous injection, and their ears were collected. The extravagated dye was then extracted from each ear with 400 μL of formamide at 70 °C overnight. The absorbance was then measured at 620 nm using an Ultrospec 3000 spectrophotometer (Amersham Pharmacia Biotech, Uppsala, Sweden). The absorbance of the dye extracted from the left ear was subtracted with that from the right ear. Statistical analysis was performed using GraphPad Prism version 7.02 (GraphPad Software, La Jolla, CA, USA). One-way analysis of variance followed by Dunnett's multiple comparison tests was used to assess the statistical significance. Values of $^*P < 0.05$ were considered statistically significant. A protocol of the PCA experiment was approved by the Animal Experiment Committee of Ehime University. All animal experiments were carried out in accordance with the Guidelines of Animal Experiments of Ehime University.

**FAAH Substrate Assay.** Transgenic expression of the human FAAH in a baculovirus expression system using Sf9 cells was performed by following a method described previously.[42] Test compounds in Table 5 diluted in 0.2 mL of 20 mM Tris buffer (pH 8.0) were incubated with Sf9 cell lysate (10 μg protein) for 60 min at 37 °C and subsequently mixed with 0.6 mL of acetonitrile. After centrifugation, the supernatant was subjected to the HPLC analysis conducted on an Acquity UPLC system (Waters) with a diode array detector. Chromatographic separations were conducted using an Acquity UPLC BEH $C_{18}$ (1.7 μm, 2.1 × 100 mm) column (Waters). The absorbance was detected at 210 nm to measure the appearance of DHA.

**FAAH Inhibition Assay.** Sensitive fluorescent-based FAAH inhibition assay was performed by using *N*-(6-methoxypyridin-3-yl)octanamide as a fluorescent substrate as described previously.[42] Test compounds mixed with Sf9 cell lysate (1 μg protein) in 0.1 mL of 20 mM Tris buffer (pH 8.0) were further mixed with 0.1 mL of 0.5 mM *N*-(6-methoxypyridin-3-yl)octanamide. The fluorescence intensity was immediately monitored for 15 min with an excitation wavelength of 302 nm and an emission wavelength of 396 nm using a microplate reader (SH-8000Lab; Corona Electric, Hitachinaka, Japan).

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.9b00994.

Molecular formula strings (CSV)

$^1$H NMR, $^{13}$C NMR, and HPLC spectra for DHEA, compounds **28**, **29**, **34**, **35**, **46**, **57**, **58**, and **59** (PDF)

## ■ AUTHOR INFORMATION


### Corresponding Author

*E-mail: nishi.kosuke.mx@ehime-u.ac.jp. Phone/Fax: +81-89-946-9818.

### ORCID

Kosuke Nishi: 0000-0003-4205-8465


### Author Contributions

I.-H.K. and K.N. conceived and designed research; I.-H.K. performed the syntheses; I.-H.K., H.N., and K.N. performed the analytical characterization of synthesized compounds; Y.K. and K.N. performed biological experiments and analyzed the data; T.S. supervised the research; I.-H.K. wrote the manuscript.

### Notes

The authors declare no competing financial interest.

## ■ ACKNOWLEDGMENTS

Animal experiments were conducted at the Division of Genetic Research of the Advanced Research Support Center (ADRES), Ehime University. This work was partially supported by JSPS KAKENHI, Grant-in-Aid for Scientific Research (C), grant number JP17K07792 (to K.N.).

## ■ ABBREVIATIONS

PUFAs, polyunsaturated fatty acids; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; DHEA, docosahexaenoyl ethanolamide; EPEA, eicosapentaenoyl ethanolamide; EDCI, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride; DMAP, 4-dimethylaminopyridine; DMF, *N*,*N*-dimethylformamide; THF, tetrahydrofuran; PCA, passive cutaneous anaphylaxis; MT buffer, modified Tyrode's buffer; DNP, dinitrophenol; HSA, human serum albumin; FAAH, fatty acid amide hydrolase

## ■ REFERENCES


(1) Moon, T. C.; Befus, A. D.; Kulka, M. Mast cell mediators: their differential release and the secretory pathways involved. *Front. Immunol.* **2014**, *5*, 569.

(2) Maeyama, K.; Hohman, R. J.; Metzger, H.; Beaven, M. A. Quantitative relationships between aggregation of IgE receptors, generation of intracellular signals, and histamine secretion in rat basophilic leukemia (2H3) cells. Enhanced responses with heavy water. *J. Biol. Chem.* **1986**, *261*, 2583−2592.

(3) Galli, S. J.; Tsai, M. IgE and mast cells in allergic disease. *Nat. Med.* **2012**, *18*, 693−704.

(4) Levy, B. D.; Kohli, P.; Gotlinger, K.; Haworth, O.; Hong, S.; Kazani, S.; Israel, E.; Haley, K. J.; Serhan, C. N. Protectin D1 is




DOI: 10.1021/acs.jmedchem.9b00994
J. Med. Chem. 2019, 62, 9576−9592

generated in asthma and dampens airway inflammation and hyperresponsiveness. *J. Immunol.* **2007**, *178*, 496−502.
(5) Platts-Mills, T. A. E. The allergy epidemics: 1870-2010. *J. Allergy Clin. Immunol.* **2015**, *136*, 3−13.
(6) Nakae, K.; Baba, K. Update on epidemiology of pollinosis in Japan: changes over the last 10 years. *Clin. Exp. Allergy Rev.* **2010**, *10*, 2−7.
(7) Nwaru, B. I.; Hickstein, L.; Panesar, S. S.; Muraro, A.; Werfel, T.; Cardona, V.; Dubois, A. E. J.; Halken, S.; Hoffmann-Sommergruber, K.; Poulsen, L. K.; Roberts, G.; Van Ree, R.; Vlieg-Boerstra, B. J.; Sheikh, A. The epidemiology of food allergy in Europe: a systematic review and meta-analysis. *Allergy* **2014**, *69*, 62−75.
(8) Meijerink, J.; Plastina, P.; Vincken, J.-P.; Poland, M.; Attya, M.; Balvers, M.; Gruppen, H.; Gabriele, B.; Witkamp, R. F. The ethanolamide metabolite of DHA, docosahexaenoylethanolamine, shows immunomodulating effects in mouse peritoneal and RAW264.7 macrophages: evidence for a new link between fish oil and inflammation. *Br. J. Nutr.* **2011**, *105*, 1798−1807.
(9) Calder, P. C. Immunomodulation by omega-3 fatty acids. *Prostaglandins, Leukotrienes Essent. Fatty Acids* **2007**, *77*, 327−335.
(10) Grigoriev, V. V.; Serkov, I. V.; Beznosko, B. K.; Ivanova, T. A.; Gretskaya, N. M.; Bezuglov, V. V.; Bachurin, S. O. Influence of derivatives of arachidonic and docosohexaenic acids on AMPA receptors in Purkinje neurons and cognitive functions in mice. *Biol. Bull.* **2010**, *37*, 312−315.
(11) Rashid, M. A.; Kim, H.-Y. N-Docosahexaenoylethanolamine ameliorates ethanol-induced impairment of neural stem cell neurogenic differentiation. *Neuropharmacology* **2016**, *102*, 174−185.
(12) Kim, H.-Y.; Moon, H.-S.; Cao, D.; Lee, J.; Kevala, K.; Jun, S. B.; Lovinger, D. M.; Akbar, M.; Huang, B. X. N-Docosahexaenoylethanolamide promotes development of hippocampal neurons. *Biochem. J.* **2011**, *435*, 327−336.
(13) Rovito, D.; Giordano, C.; Vizza, D.; Plastina, P.; Barone, I.; Casaburi, I.; Lanzino, M.; De Amicis, F.; Sisci, D.; Mauro, L.; Aquila, S.; Catalano, S.; Bonofiglio, D.; Andò, S. Omega-3 PUFA ethanolamides DHEA and EPEA induce autophagy through PPAR$\gamma$ activation in MCF-7 breast cancer cells. *J. Cell. Physiol.* **2013**, *228*, 1314−1322.
(14) Serini, S.; Fasano, E.; Piccioni, E.; Cittadini, A. R. M.; Calviello, G. Dietary n-3 polyunsaturated fatty acids and the paradox of their health benefits and potential harmful effects. *Chem. Res. Toxicol.* **2011**, *24*, 2093−2105.
(15) Brown, I.; Cascio, M. G.; Wahle, K. W. J.; Smoum, R.; Mechoulam, R.; Ross, R. A.; Pertwee, R. G.; Heys, S. D. Cannabinoid receptor-dependent and -independent anti-proliferative effects of omega-3 ethanolamides in androgen receptor-positive and -negative prostate cancer cell lines. *Carcinogenesis* **2010**, *31*, 1584−1591.
(16) Mimeault, M.; Pommery, N.; Wattez, N.; Bailly, C.; Henichart, J. P. Anti-proliferative and apoptotic effects of anandamide in human prostatic cancer cell lines: implication of epidermal growth factor receptor down-regulation and ceramide production. *Prostate* **2003**, *56*, 1−12.
(17) Berger, A.; Crozier, G.; Bisogno, T.; Cavaliere, P.; Innis, S.; Di Marzo, V. Anandamide and diet: inclusion of dietary arachidonate and docosahexaenoate leads to increased brain levels of the corresponding N-acylethanolamines in piglets. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 6402−6406.
(18) (a) Willemsen, L. E. M. Dietary n-3 long chain polyunsaturated fatty acids in allergy prevention and asthma treatment. *Eur. J. Pharmacol.* **2016**, *785*, 174−186. (b) Barros, R.; Moreira, A.; Padrão, P.; Teixeira, V. H.; Carvalho, P.; Delgado, L.; Lopes, C.; Severo, M.; Moreira, P. Dietary patterns and asthma prevalence, incidence and control. *Clin. Exp. Allergy* **2015**, *45*, 1673−1680.
(19) Lumia, M.; Luukkainen, P.; Tapanainen, H.; Kaila, M.; Erkkola, M.; Uusitalo, L.; Niinistö, S.; Kenward, M. G.; Ilonen, J.; Simell, O.; Knip, M.; Veijola, R.; Virtanen, S. M. Dietary fatty acid composition during pregnancy and the risk of asthma in the offspring. *Pediatr. Allergy Immunol.* **2011**, *22*, 827−835.
(20) Miyake, Y.; Sasaki, S.; Tanaka, K.; Ohya, Y.; Miyamoto, S.; Matsunaga, I.; Yoshida, T.; Hirota, Y.; Oda, H. Fish and fat intake and prevalence of allergic rhinitis in Japanese females: the Osaka Maternal and Child Health Study. *J. Am. Coll. Nutr.* **2007**, *26*, 279−287.
(21) Kim, I.-H.; Park, Y.-K.; Nishiwaki, H.; Hammock, B. D.; Nishi, K. Structure-activity relationships of amide-phosphonate derivatives as inhibitors of the human soluble epoxide hydrolase. *Bioorg. Med. Chem.* **2015**, *23*, 7199−7210.
(22) Yamano, Y.; Tsuboi, K.; Hozaki, Y.; Takahashi, K.; Jin, X.-H.; Ueda, N.; Wada, A. Lipophilic amines as potent inhibitors of N-acylethanolamine-hydrolyzing acid amidase. *Bioorg. Med. Chem.* **2012**, *20*, 3658−3665.
(23) Hernández, J. N.; Ramírez, M. A.; Martín, V. S. A New Selective Cleavage ofN,N-Dicarbamoyl-Protected Amines Using Lithium Bromide. *J. Org. Chem.* **2003**, *68*, 743−746.
(24) Li, J.; Sha, Y. A convenient synthesis of amino acid methyl esters. *Molecules* **2008**, *13*, 1111−1119.
(25) Suseno, S.; Hayati, S.; Izaki, A. Fatty acid composition of some potential fish oil from production centers in Indonesia. *Orient. J. Chem.* **2014**, *30*, 975−980.
(26) Molla, R.; Asaduzzaman, A. K. M.; Mia, A. R.; Zeb, A.; Uddin, S. Extraction and characterization of oil and lecithin from Boal (Wallago attu) fish. *J. Food Nutr. Res.* **2015**, *3*, 661−666.
(27) Schwartz, L. B.; Lewis, R. A.; Seldin, D.; Austen, K. F. Acid hydrolases and tryptase from secretory granules of dispersed human lung mast cells. *J. Immunol.* **1981**, *126*, 1290−1294.
(28) MacDonald, A. J.; Haig, D. M.; Bazin, H.; McGuigan, A. C.; Moqbel, R.; Miller, H. R. IgE-mediated release of rat mast cell protease II, $\beta$-hexosaminidase and leukotriene $C_4$ from cultured bone marrow-derived rat mast cells. *Immunology* **1989**, *67*, 414−418.
(29) van den Elsen, L. W. J.; Nusse, Y.; Balvers, M.; Redegeld, F. A.; Knol, E. F.; Garssen, J.; Willemsen, L. E. M. n-3 Long-chain PUFA reduce allergy-related mediator release by human mast cells in vitro via inhibition of reactive oxygen species. *Br. J. Nutr.* **2013**, *109*, 1821−1831.
(30) Cruz, S. L.; Sánchez-Miranda, E.; Castillo-Arellano, J. I.; Cervantes-Villagrana, R. D.; Ibarra-Sánchez, A.; González-Espinosa, C. Anandamide inhibits Fc$\varepsilon$RI-dependent degranulation and cytokine synthesis in mast cells through $CB_2$ and GPR55 receptor activation. Possible involvement of $CB_2$-GPR55 heteromers. *Int. Immunopharmacol.* **2018**, *64*, 298−307.
(31) Nishi, K.; Teranishi, M.; Yasunaga, S.; Iitsuka, M.; Matsumoto, S.; Sugahara, T. The major whey protein $\beta$-lactoglobulin inhibits IgE-mediated degranulation of RBL-2H3 cells and passive cutaneous anaphylaxis in mice. *Int. Dairy J.* **2014**, *39*, 89−95.
(32) Finkelman, F. D. Anaphylaxis: lessons from mouse models. *J. Allergy Clin. Immunol.* **2007**, *120*, 506−515.
(33) Kono, R.; Nakamura, M.; Nomura, S.; Kitano, N.; Kagiya, T.; Yoshiharu, O.; Inada, K.; Tokuda, A.; Utsunomiya, H.; Ueno, M. Biological and epidemiological evidence of anti-allergic effects of traditional Japanese food ume (Prunus mume). *Sci. Rep.* **2018**, *8*, 11638.
(34) Yang, R.; Fredman, G.; Krishnamoorthy, S.; Agrawal, N.; Irimia, D.; Piomelli, D.; Serhan, C. N. Decoding functional metabolomics with docosahexaenoyl ethanolamide (DHEA) identifies novel bioactive signals. *J. Biol. Chem.* **2011**, *286*, 31532−31541.
(35) McDougle, D. R.; Watson, J. E.; Abdeen, A. A.; Adili, R.; Caputo, M. P.; Krapf, J. E.; Johnson, R. W.; Kilian, K. A.; Holinstat, M.; Das, A. Anti-inflammatory $\omega$-3 endocannabinoid epoxides. *Proc. Natl. Acad. Sci. U.S.A.* **2017**, *114*, No. E6034.
(36) Meijerink, J.; Balvers, M.; Witkamp, R. N-acyl amines of docosahexaenoic acid and othern-3 polyunsatured fatty acids - from fishy endocannabinoids to potential leads. *Br. J. Pharmacol.* **2013**, *169*, 772−783.
(37) Brown, I.; Cascio, M. G.; Wahle, K. W. J.; Smoum, R.; Mechoulam, R.; Ross, R. A.; Pertwee, R. G.; Heys, S. D. Cannabinoid receptor-dependent and -independent anti-proliferative effects of omega-3 ethanolamides in androgen receptor-positive and -negative prostate cancer cell lines. *Carcinogenesis* **2010**, *31*, 1584−1591.
(38) Björklund, E.; Blomqvist, A.; Hedlin, J.; Persson, E.; Fowler, C. J. Involvement of fatty acid amide hydrolase and fatty acid binding protein 5 in the uptake of anandamide by cell lines with different levels


DOI: 10.1021/acs.jmedchem.9b00994
*J. Med. Chem.* 2019, 62, 9576−9592

of fatty acid amide hydrolase expression: a pharmacological study. *PLoS One* **2014**, *9*, No. e103479.

(39) Facci, L.; Dal Toso, R.; Romanello, S.; Buriani, A.; Skaper, S. D.; Leon, A. Mast cells express a peripheral cannabinoid receptor with differential sensitivity to anandamide and palmitoylethanolamide. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 3376−3380.

(40) Lee, J.-W.; Huang, B. X.; Kwon, H.; Rashid, M. A.; Kharebava, G.; Desai, A.; Patnaik, S.; Marugan, J.; Kim, H. Y. Orphan GPR110 (ADGRF1) targeted by N-docosahexaenoylethanolamine in development of neurons and cognitive function. *Nat. Commun.* **2016**, *7*, 13123.

(41) Watanabe, J.; Shinmoto, H.; Tsushida, T. Coumarin and flavone derivatives from estragon and thyme as inhibitors of chemical mediator release from RBL-2H3 cells. *Biosci., Biotechnol., Biochem.* **2005**, *69*, 1−6.

(42) Huang, H.; Nishi, K.; Tsai, H.-J.; Hammock, B. D. Development of highly sensitive fluorescent assays for fatty acid amide hydrolase. *Anal. Biochem.* **2007**, *363*, 12−21.



DOI: 10.1021/acs.jmedchem.9b00994
*J. Med. Chem.* 2019, 62, 9576−9592