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Structure–activity relationships of vanillic acid ester analogs in inhibitory effect of antigen-mediated degranulation in rat basophilic leukemia RBL-2H3 cells

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ABSTRACT

Methyl vanillate (**1**) showed strong degranulation inhibitory activity among vanillin derivatives tested. In order to find structure–activity relationships for developing anti-allergic agents with simple structures and potent activity, we synthesized several vanillic acid (VA) ester derivatives with C_1–C_4 and C_8 alkyl chains and evaluated their degranulation inhibitory activities. The most active compound of VA ester derivatives was derivative **5** with a C_4 straight alkyl chain, and derivative **5** exhibited approximately three-fold greater inhibitory activity than that of **1**. Moreover, we designed 8 types of analogs based on **5**, and we found that the minimum structure for potent degranulation inhibitory activity requires direct connection of the butyl ester moiety on the benzene ring and at least one hydroxyl group on the benzene ring. Butyl *meta* or *para* hydroxyl benzoate (**10** or **11**) has a simpler structure than that of **5** and exhibited more potent degranulation inhibitory activity than that of **5**.

Epidemiology has suggested that the prevalence of atopic dermatitis has increased over the past 30 years, and similar trends have been reported for asthma and hay fever.[1] These diseases are classified as immediate hypersensitivity (type I) allergy. The increasing prevalence of type I allergies has become a social problem, especially in developed countries. Type I allergy is caused by the cross-linking of high-affinity receptors for IgE (FcεRI) following IgE. The IgE-FcεRI complex with a specific antigen leads to the release of chemical mediators such as histamine, arachidonic acid metabolites, proteases, serotonin, cytokines and heparin.[2–5] This phenomenon is called 'degranulation', and allergic reaction is caused by degranulation.

Recently, several drugs and natural products have been analyzed and have been shown to inhibit degranulation via suppression of signal pathways, suggesting that they could be valuable compounds for allergy therapy.[6–12] However, the chemical structures are complex, and it is not clear how the structure affects degranulation inhibitory activity. Even if the active body is revealed, it would be difficult to produce anti-allergic drugs with a complex chemical structure on an industrial scale. The development of an anti-allergic agent that has a simple chemical structure and shows potent degranulation inhibitory activity is needed. We focused on vanillic acid (VA) and its derivatives as compounds with simple chemical structures. These compounds naturally occur in plants. There have been several reports showing that they have multiple physiological functions including antioxidative activity,[13,14] anti-inflammatory effect[15] and restorative effect on a primary CoQ deficiency.[16] As for immune functions, VA has been reported to have inhibitory activity against ulcerative colitis[17] and hepatoprotective activity[18] by suppressing immune mediators such as interleukin-6, interferon-γ and tumor necrosis factor-α. Although VA and its derivatives have been shown to have multiple physiological functions, there has been no report about their anti-allergy activity. In this study, we investigated chemical structures that have potent degranulation inhibitory activity by screening several derivatives and analogs based on the structure of VA.

Firstly, we evaluated anti-degranulation activity using vanillin (VN), VA and methyl vanillate (**1**), which are contained in many plants and are classified as phenolic compounds. Oxatomide, which is clinically used as an anti-allergy drug, was used as a positive control for the assay. As a result, compound **1** at a concentration of 1000 μM showed inhibitory activity against chemical mediator release from RBL-2H3, but VN and VA did not (Fig. 1). Ferulic acid, which is metabolized to vanillin and vanillic acid, had less anti-degranulation activity than did **1** (data not shown). These results suggested that the relatively lipophilic VA ester had more potent inhibitory degranulation activity than that of

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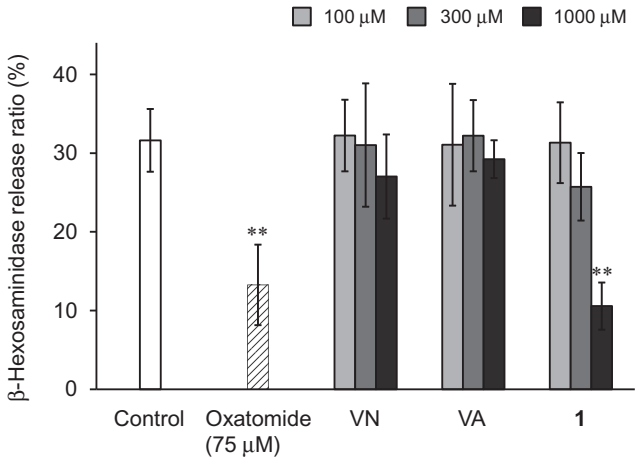


Figure 1. Inhibitory effects of VN, VA and **1** on antigen-induced degranulation in RBL-2H3 cells. DNP-IgE-sensitized RBL-2H3 cells were incubated with 75 μM oxatomide for a positive control or with indicated concentrations of these samples for 20 min and stimulated with DNP-HSA for 1 h. Release of β-hexosaminidase was measured. Each value is the mean of three independent cultures, and the bars show S.D. $^{**}p < 0.01$ (Dunnett's test) as compared with the control.

hydrophilic VN and VA in the anti-degranulation assay. It has been reported that membrane permeability and metabolic rate vary depending on the type (straight or branched) or length of lipophilic chains.[19–21] Hence, degranulation inhibitory activity of a series of VA esters possessing ester chains of varying lengths from C_1 to C_4 and C_8 was investigated (Fig. 2A). VA ester derivatives **3**, **4** and **6** were obtained by coupling VA and the corresponding alcohol by using acetyl chloride as an acid catalyst (see Supplementary data). The structures of all of the compounds were confirmed by spectroscopies (^{1}H and ^{13}C NMR and mass spectrometry, see Supplementary data). With increasing ester chain length, VA esters generally tended to have poor solubility in the culture medium. To solubilize the compounds, α-cyclodextrin (α-CD) was used in this assay. α-CD has been reported to have an 'inclusion effect', and this effect increases the solubility of guest molecules.[22] All of the compounds were dissolved in a culture medium containing α-CD (final concentration of 300 μM). α-CD at a concentration of 300 μM has been shown not to affect the activity of VA esters in this assay.

The degranulation inhibitory activity of VA ester derivatives possessing straight chains from C_1 to C_4 tended to increase with increasing length of their ester group (Fig. 2B). Derivative **5** with a C_4 straight chain (IC_{50} = 198 μM) showed approximately three-fold higher inhibitory activity than that of original compound **1** with a C_1 chain (IC_{50} = 640 μM). In contrast, a branched-chain ester had lower activity than that of a straight-chain ester. Actually, the potency of derivative **4** with a C_3 branched chain (IC_{50} = 415 μM) was approximately 30% lower than that of derivative **3** possessing a C_3 straight chain (IC_{50} = 294 μM). The VA ester with the longest chain, derivative **6**, increased degranulation caused by cytotoxicity (data not shown). Thus, the results of this assay revealed that derivative **5** had suitable lipophilicity, water solubility and potent degranulation inhibitory activity without cytotoxicity. It is possible that VA ester derivatives taken into the cells were cleaved by the cell surface esterase or intracellular esterase and the resulting metabolite mixture exhibited inhibitory activity. However, metabolites of **5**, VA and butanol did not show an inhibitory effect (data not shown). Considering these data, it seems that VA esters showed activity at the cell surface and/or in the intracellular compartment before hydrolysis of VA esters by esterase and that the lipophilic side chain of VA esters played an important role in the membrane permeability and distribution of VA esters in mast cells.

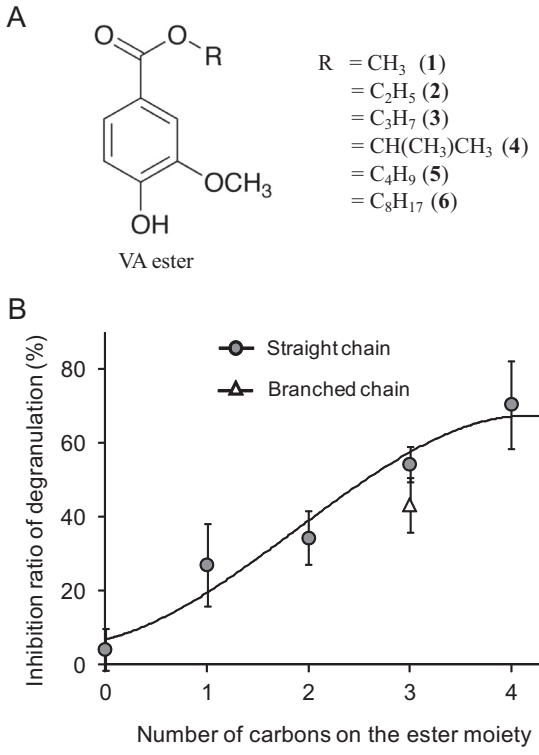
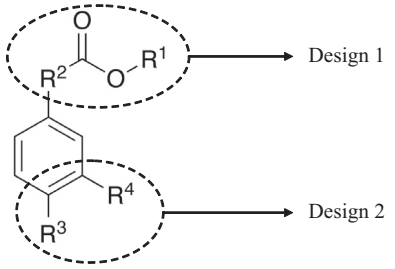


Figure 2. Structures and degranulation inhibitory activity of VA esters. (A) Structures of VA esters. (B) Influence of the ester moiety of VA ester on degranulation inhibitory activity. The data show the inhibition ratio of degranulation in each ester group at 300 μM. Each value is the mean of three independent cultures, and the bars show SD.

Table 1
Structures of synthesized compounds for designs 1 and 2



Compound	Design			
	1		2	
	R^1	R^2	R^3	R^4
7	C_3H_7	CH_2	OH	OCH_3
8	C_2H_5	C_2H_4	OH	OCH_3
9	C_4H_9	–	OCH_3	OH
10	C_4H_9	–	OH	H
11	C_4H_9	–	H	OH
12	C_4H_9	–	OCH_3	H
13	C_4H_9	–	H	OCH_3
14	C_4H_9	–	H	H

The results indicated that derivative **5** per se with a C_4 straight-chain ester exhibited degranulation inhibitory activity.

VA esters have a hydroxyl group at the *para* position and a methoxy group at the *meta* position on the benzene ring and are compounds having an ester group as the lipophilic moiety. It was investigated whether the position of the oxycarbonyl group in the lipophilic chain was important for exhibiting degranulation

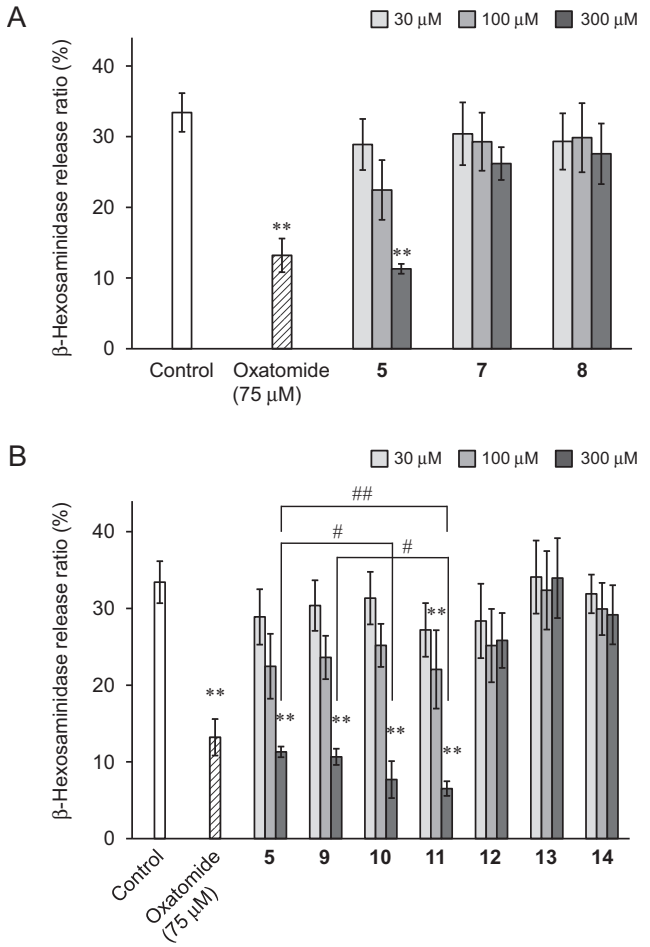


Figure 3. Inhibitory effects of **5** analogs on antigen-induced degranulation in RBL-2H3 cells. (A) Degranulation inhibitory activity of analogs **7** and **8** in design 1. (B) Degranulation inhibitory activity of analogs **9**–**14** in design 2. DNP-IgE-sensitized RBL-2H3 cells were incubated with 75 μM oxatomide for a positive control or with indicated concentrations of these samples for 20 min and stimulated with DNP-HSA for 1 h. Release of β-hexosaminidase was measured. Each value is the mean of three independent cultures, and the bars show SD. $^{**}p < 0.01$ (Dunnett's test) as compared with the control. $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ (Student's *t*-test) as compared with **5** or **9**.

inhibitory activity and whether the type of functional group on the benzene ring was important for exhibiting the activity. We propose two kinds of design for developing structure–activity relationships (Table 1). Synthetic methods are shown in Supplementary data. In design 1, VA ester analogs **7** and **8**, in which the position of the ester group in the lipophilic chain varied, were designed. In design 2, VA ester analogs **9**–**14**, in which the types of functional groups at the *para* and/or *meta* positions on the benzene ring were changed, were designed. These VA ester analogs were designed on the basis of derivative **5**, which showed the most potent degranulation inhibitory activity among VA ester derivatives **1**–**6**.

In design 1, the position of the ester group was changed from the benzoic acid group to phenylacetic acid and phenylpropionic acid groups (**7** and **8**) in order to investigate whether only the lipophilicity of the ester group was necessary for the role in membrane permeability and distribution or whether the structure of the ester group was involved in the degranulation inhibitory activity. When the ester moiety was not directly connected to the benzene ring (**7** and **8**), the inhibitory degranulation activity was abolished (Fig. 3A). This result suggested that the resonance structure between the benzene ring and oxycarbonyl group interacted with the factor of degranulation. Actually, it has been reported that the activity of scavenging degranulation factor was related to electron density on the benzene ring.[23] Therefore, this result indicates that the ester structure itself of VA esters, an oxycarbonyl group directly connected to the benzene ring, is a critical factor for inhibition of the degranulation in mast cells.

In design 2, structure–activity relationships of functional groups on the benzene ring were investigated (Fig. 3B). When the positions of the hydroxyl and methoxy groups on the benzene ring of derivative **5** were changed, the degranulation inhibitory activity of **9** showed the same tendency as that of **5**. However, removal of all functional groups (**14**) resulted in loss of the inhibitory activity. These results suggested that functional groups on the benzene ring were essential for the degranulation inhibitory activity. When only the hydroxyl group on the benzene ring was in the *para* or *meta* position, analogs **10** and **11** showed greater degranulation inhibitory activities than those of **5** and **9** with the methoxy group and hydroxyl group on the benzene ring. On the other hand, degranulation inhibitory activity of analogs **12** and **13** methylated to the hydroxyl group of the analogs **10** and **11** was abolished, suggesting that the methoxy group on the benzene ring of VA esters inhibited their activity. From these results, it was found that a simple structure with a hydroxyl benzoic acid ester such as paraben or *m*-hydroxy benzoate showed potent degranulation inhibitory activity.

Parabens have been reported to cause allergic contact dermatitis.[24] Indeed, parabens with a C_{7-10} side chain showed significant histamine release in vitro and heptylparaben elicited a strong skin reaction in vivo, but parabens with a C_{1-4} side chain caused no significant degranulation and butylparaben did not induced a strong skin reaction. It was also reported that methyl paraben possibly has some inhibitory effects on histamine release.[25] Although parabens have been reported to cause allergic contact dermatitis, degranulation might be inhibited by a paraben with adequate lipophilicity of the alkyl side chain. Our data agree with data in those previous reports. The VA ester with the longest chain, derivative **6**, showed cytotoxicity and increased degranulation as result, although VA esters with a C_{1-4} chain inhibited degranulation. It is thought that allergic contact dermatitis induced by paraben with a heavy side chain is indirectly caused by cytotoxicity. Therefore, these results of the structure–activity relationship study revealed that directly connecting the butyl ester moiety on the benzene ring was required for potent degranulation inhibitory activity and that at least one hydroxyl group on the benzene ring was required for the activity.

In conclusion, derivative **5** with a C_4 straight-chain ester had the most potent degranulation inhibitory activity among the VA ester derivatives, and it exhibited approximately three-fold greater inhibitory activity than that of original compound **1**. Moreover, analog **10** demethoxylated from derivative **5** and its isomer (**11**) had significantly potent activity in comparison with that of derivative **5**. From the results of study on structure–activity relationships of VA esters, we found that the minimum structure for potent degranulation inhibitory activity required direct connection of butyl ester moiety on the benzene ring and at least one hydroxyl group on the benzene ring. The results of this study are expected to contribute to the development of novel anti-allergic drugs with simple structures.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2016.06.028.

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