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Antiallergic Activity Profile in Vitro RBL-2H3 and in Vivo Passive Cutaneous Anaphylaxis Mouse Model of New Sila-Substituted 1,3,4-Oxadiazoles

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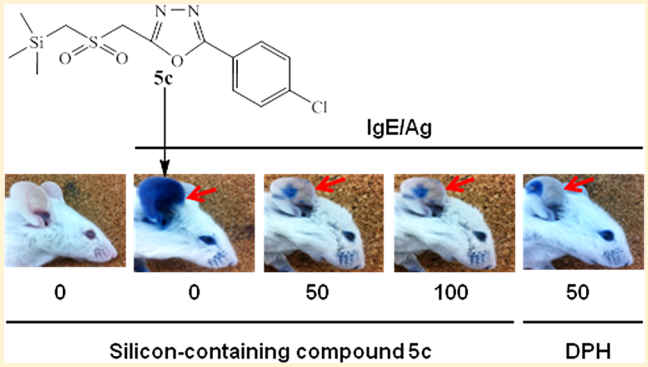
Supporting Information

ABSTRACT: A new class of sila-substituted 1,3,4-oxadiazoles was synthesized and evaluated for antiallergic activity using RBL-2H3 as the in vitro model and the in vivo anaphylactic mouse model. We observed that compound **5c** effectively suppressed DNP-HSA-induced mast cell degranulation, compared to carbon analogue **9**, and also suppressed the expression of TNF-α mRNA and Akt phosphorylation in antigen-stimulated RBL-2H3 cells. We also studied the effect of **5c** in an in vivo passive cutaneous anaphylaxis (PCA) mouse model. The suppression by **5c** was more effective than that by diphenylhydramine (DPH), a typical anti-histamine drug.



INTRODUCTION

Day-by-day allergy disease increases drastically throughout the world because of host (host factors include heredity, gender, race, and age) and environmental factors.[1] Allergy is an immunological disorder that occurs when the immune system reacts to normally harmless substances in the environment. Most of the allergic reactions are distinctive because of excessive activation of certain mast cells and basophils by an antibody immunoglobulin E (IgE).[2] Mast cells are central to the pathogenesis of immediate hypersensitivity, and they are located in blood vessels and at epithelial surfaces such as the lung and intestine. Such epithelial location makes mast cells one of the first activators of the inflammatory response.[3−5] Mast cells have a high affinity for IgE because of having a specific IgE receptor, FcεR1, on their surface. As the IgE−multivalent allergen complex binds to FcεR1, the mast cells become activated and release a range of preformed species such as newly synthesized mediators and cytokines that trigger potent allergic reactions.[6] In normal conditions, the mast cells have preformed mediators such as histamine serine proteases and β-hexosaminidase in their cytoplasmic granules. The activated mast cells release these mediators in response to immune allergic reactions through IgE−FcεR1 binding.[3−9]

β-Hexosaminidase, a granule-associated exoglycosidase, has been used to monitor mast cell degranulation just as histamine has been used. β-Hexosaminidase could act with tryptases and chymases to induce degradation of the extracellular matrix. This extracellular matrix degradation is an important event during the remodeling of inflamed tissue.[10]

Recently we have focused on the syntheses of sila-substituted five-membered ring heterocycles such as 1,3,4-oxadiazoles, 1,3,4-thiadiazoles, 1,2,4-triazoles, pyrazoles, isoxazoles, and imidazoles because these compounds would display broad spectra of biological activities. Particularly various substituted 1,3,4-oxadiazoles have been much explored for their biological activities such as antiallergic,[11] antiviral,[12] anti-inflammatory,[13] anticancer,[14] antibacterial,[15] and antifungal agents.[15]

Bioorganosilicon chemistry is known to be a powerful tool for the generation of new therapeutic agents. Up to now several silicon-containing drugs have been reported in the literature; those are polydimethylsiloxanes(I),[16] cyclohexyl(phenyl)(3-(piperidin-1-yl)propyl)silanol(II),[17] sila-derived phthalocyanine(III),[18] etc. (Figure 1). Recently some of the silicon-containing drugs have entered human clinical trials; those are silperisone,[19] Tac101,[20] BNP 1350,[21] etc. In this context, we have synthesized various sila-substituted 1,3,4-oxadiazoles and examined their antiallergic effect using RBL-2H3 as the in vitro model and the in vivo anaphylactic mouse model. Our results indicate that silicon-containing compound **5c** not only effectively inhibits the degranulation and expression of cytokines involved in type 1 hypersensitivity in mast cells but

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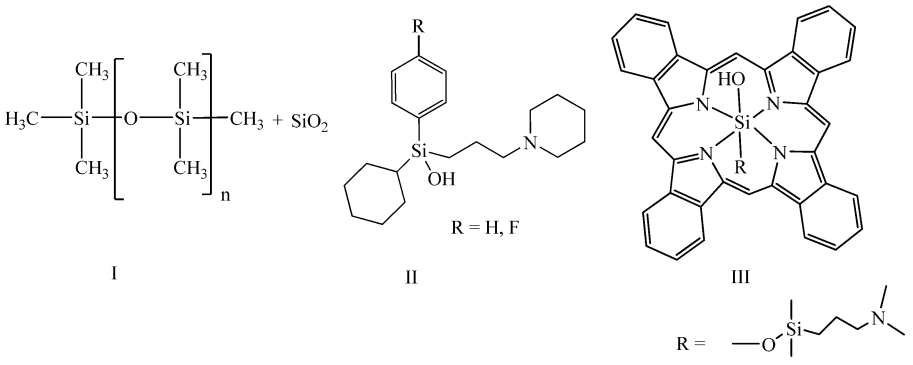


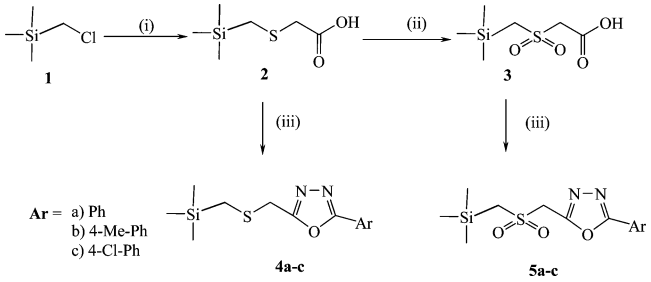
Figure 1. Biologically active organosilicon compounds.

also suppresses hypersensitive reactions in the PCA mouse model.

RESULTS AND DISCUSSION

Chemistry. The sila-substituted thio/sulfone linked 1,3,4-oxadiazoles were synthesized in high yields. The synthetic intermediate 2-(((trimethylsilyl)methylthio)acetic acid (**2**) was prepared by the reaction of (chloromethyl)trimethylsilane (**1**) with mercaptoacetic acid in the presence NaOH/MeOH. The oxidation of **2** in the presence of H_2O_2/CH_3COOH[22] at 0 °C resulted in 2-((trimethylsilyl)methylsulfonyl)acetic acid (**3**). The desired heterocycles 2-aryl-5-(((trimethylsilyl)methylthio)methyl)-1,3,4-oxadiazoles (**4a–c**) and 2-aryl-5-(((trimethylsilyl)methylsulfonyl)methyl)-1,3,4-oxadiazoles (**5a–c**) were synthesized respectively by the reaction of **2** and **3** with the corresponding benzohydrazides in the presence of phosphorus oxychloride[23] (Scheme 1).

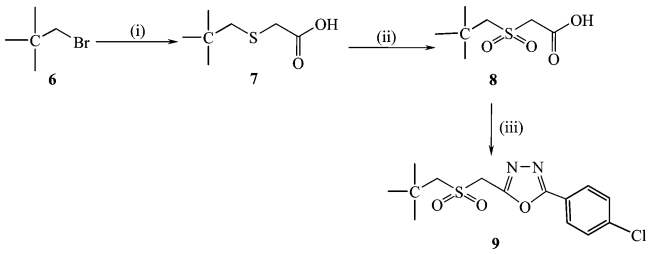
Scheme 1. Synthesis of 2-Aryl-5-(((trimethylsilyl)methylthio)methyl)-1,3,4-oxadiazoles 4 and 2-Aryl-5-(((trimethylsilyl)methylsulfonyl)methyl)-1,3,4-oxadiazoles 5[a]



[a]Reagents and conditions: (i) $HSCH_2COOH$, MeOH, NaOH, reflux, 4 h; (ii) 30% H_2O_2, AcOH, 0 °C, 24 h; (iii) $ArCONHNH_2$, $POCl_3$, reflux, 4–6 h.

For the comparison of antiallergic activity to **5c**, we synthesized 2-(4-chlorophenyl)-5-(neopentylsulfonylmethyl)-1,3,4-oxadiazole (**9**) as a carbon analogue[24] of it. Compound **9** was synthesized as follows: 2-(neopentylthio)acetic acid was synthesized from the reaction of neopentyl bromide (neopentyl chloride did not work) with mercaptoacetic acid followed by oxidation with H_2O_2/CH_3COOH[22] at 0 °C to give 2-(neopentylsulfonyl)acetic acid. Finally, 2-(neopentylsulfonyl)-acetic acid was treated with 4-chlorobenzohydrazide in the presence of phosphorus oxychloride[23] to yield target compound **9** (Scheme 2).

Scheme 2. Synthesis of 2-(4-Chlorophenyl)-5-(neopentylsulfonylmethyl)-1,3,4-oxadiazole 9[a]



[a]Reagents and conditions: (i) $HSCH_2COOH$, EtOH, KOH, reflux, 8 h; (ii) 30% H_2O_2, AcOH, 0 °C, 24 h; (iii) 4-Cl-$PhCONHNH_2$, $POCl_3$, reflux, 5 h.

Effect of Silicon-Containing Compounds on β-Hexosaminidase Release in IgE/Ag-Sensitized RBL-2H3 Cells. The RBL-2H3 cells were activated when the IgE/Ag complex bound their receptor, FcεR1. The aggregation of FcεR1 by the IgE/Ag complex induced mast cell degranulation and the release of mediators and cytokines.[25−28] β-Hexosaminidase, one of these mediators, is a marker of mast cell degranulation.[29] To determine the antidegranulation effect of silicon-containing compounds, we measured the level of β-hexosaminidase release in IgE/Ag sensitized RBL-2H3 cells. We pretreated the RBL-2H3 cells with 200 μM silicon-containing compounds (**2–5**) for 30 min followed by activation with DNP-HSA (50 ng/mL) for 15 min. Among the silicon-containing compounds, **5c** significantly inhibited IgE/Ag-induced mast cell degranulation (Figure 2) and also effectively suppressed IgE/Ag-induced mast cell degranulation in a dose-dependent manner. Interestingly, **5c** showed much better suppression results than its carbon analogue **9** (Figure 3). This result strongly indicates that the silyl group in **5c** plays an important role for the effective suppression of IgE/Ag-induced mast cell degranulation. This would be due to the properties of the silicon moiety having larger molecular size, lower electronegativity, and greater lipophilicity than those of the carbon analogue. Silicon–carbon bonds (Si–C, 1.87 Å) are longer by 0.33 Å than carbon–carbon bonds (C–C, 1.54 Å), which may change the molecule's interaction with a receptor and enhance the pharmacological selectivity and potency. In general, silicon containing compounds show greater lipophilicity than carbon analogues, which might enhance tissue distribution.[30,31] Considering those effects above, **5c** is anticipated as a better antiallergy candidate than the carbon analogue.

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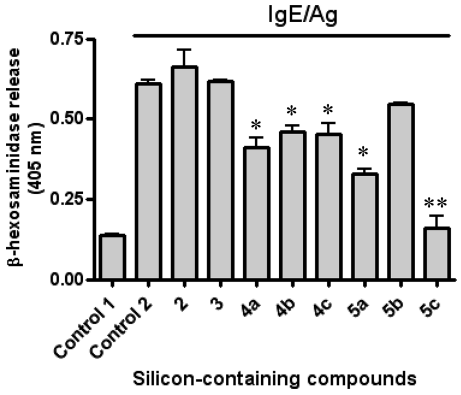

Figure 2. Effect of silicon-containing compounds on IgE/Ag-stimulated mast cell degranulation. The cells were incubated overnight in 24-well plates with 200 ng/mL DNP-specific IgE. The medium was replaced with PIPES buffer containing 100 μM silicon-containing compounds, and the cells were then challenged with 25 ng/mL DNP-HSA. After 15 min, β-hexosaminidase release was determined from the absorbance measured at 405 nm. Data are expressed as the mean ± SD (n = 4). Significant differences with the IgE/Ag-stimulated controls without silicon-containing compounds are indicated: (*) $p < 0.01$ and (**) $p < 0.001$. Control 1 is IgE/Ag(DNP-HAS)-unstimulated control without compounds. Control 2 is IgE/Ag(DNP-HAS)-stimulated control without compounds.

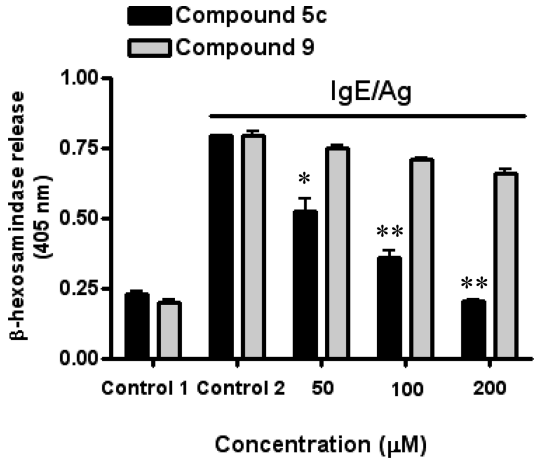

Figure 3. Effect of silicon-containing compounds on IgE/Ag-stimulated mast cell degranulation. The cells were incubated overnight in 24-well plates with 200 ng/mL DNP-specific IgE. The medium was replaced with PIPES buffer containing the indicated concentrations of silicon-containing compound **5c** and carbon analogue compound **9**, and the cells were then challenged with 25 ng/mL DNP-HSA. After 15 min, β-hexosaminidase release was determined from the absorbance measured at 405 nm. Data are expressed as the mean ± SD (n = 4). Significant differences with the IgE/Ag-stimulated controls without silicon-containing compound **5c** or carbon analogue compound **9** are indicated: (*) $p < 0.01$ and (**) $p < 0.001$. Control 1 is IgE/Ag-unstimulated control without compounds **5c** and **9**. Control 2 is IgE/Ag-stimulated control without compounds **5c** and **9**.

To confirm that the degranulation inhibition was indeed due to the activity of **5c** and its noncytotoxicity, we assessed the cytotoxicity of **5c** in the RBL-2H3 cells using an Ez-cytox assay. We pretreated the mast cells overnight with different doses of silicon-containing compound **5c**. After 23 h, cells were treated with the Ez-cytox solution for 1 h, and the absorbance of the cell culture plate was measured with a microplate reader. Silicon containing compound **5c** was noncytotoxic in the RBL-2H3 cell line at concentrations up to 200 μM (Figure 4). These findings indicate that the reduction in degranulation of the activated

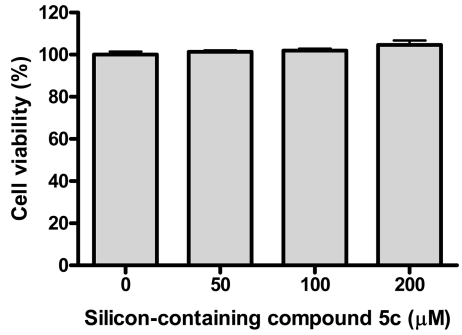

Figure 4. Cytotoxic effects of silicon-containing compound **5c** on mast cell. To assess cytotoxicity, RBL-2H3 cells were incubated overnight in medium containing 200 ng/mL DNP-specific IgE and then treated with compound **5c** at various concentrations for 23 h. Cell viability was determined with the Ez-Cytox kit from the absorbance at 450 nm.

RBL-2H3 cells was a result of the properties of the silicon-containing compound **5c** and not cytotoxicity.

Effect of Silicon-Containing Compound 5c on TNF-α mRNA Expression Level and Akt Phosphorylation in IgE/Ag-Sensitized RBL-2H3 Cells. Aggregation of IgE-bound FcεRI by Ag binding induces various inflammatory cytokines, notably TNF-α, which mediate the symptoms in an allergic reaction.[32] We treated the RBL-2H3 cells with IgE (25 ng/mL) for 24 h followed by treatment with silicon-containing compound **5c** at different concentrations (0–200 μM). The cells were then activated with DNP-HSA (25 ng/mL) for 5 min. We observed that **5c** diminished the mRNA expression levels of TNF-α in a dose-dependent manner (Figure 5). From

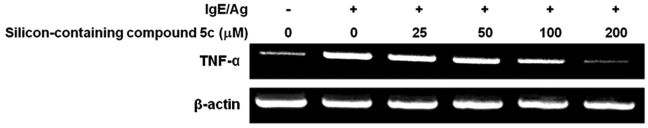

Figure 5. Effect of compound **5c** on the expression of TNF-α in IgE/Ag-stimulated mast cells. The IgE/Ag-sensitized RBL-2H3 cells were treated with various doses of silicon-containing compound **5c** and then challenged with 25 ng/mL DNP-HSA for 1 h. Total RNA was isolated and analyzed by RT-PCR.

these findings, we inferred that the antiallergic effect of **5c** was caused by suppression of the mRNA expression of these cytokines. We also examined the effects of **5c** on phosphatidylinositol 3-kinase[32] because of their role in the production of TNF-α. The phosphorylation of Akt, a surrogate for the activation of phosphatidylinositol 3-kinase, was also significantly suppressed by **5c** in a dose-dependent manner (Figure 6).

5c Reduced Anaphylactic Shock in an in Vivo Mouse Model. On the basis of good results from the in vitro model, we furthermore examined the effect of **5c** in an in vivo allergic mouse model.[33] We induced anaphylaxis by injecting IgE intradermally into one ear of each mouse. One day after IgE administration, we injected a DNP-HSA-Evans blue mixture into tail veins of each mouse to induce a systemic allergic reaction. The IgE/Ag-sensitized mouse ears subsequently swelled and turned blue. However, in the mice that received silicon-containing compound **5c** orally, the intensity of the blue

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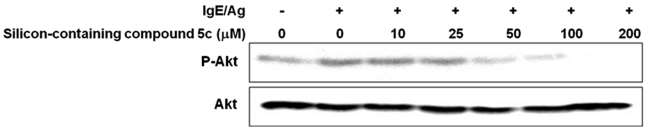

Figure 6. Effect of silicon-containing compound **5c** on Akt phosphorylation in IgE/Ag-stimulated mast cells. The IgE/Ag-sensitized RBL-2H3 cells were treated with various doses of silicon-containing compound **5c** and then challenged with 25 ng/mL DNP-HSA for 1 h. Phosphorylation of Akt was analyzed by Western blotting using specific antibodies.

dye decreased with increasing **5c** concentration. **5c** also appeared to suppress anaphylaxis in the mice, as evidenced by the results depicted in the bar graph in Figure 7. The effect

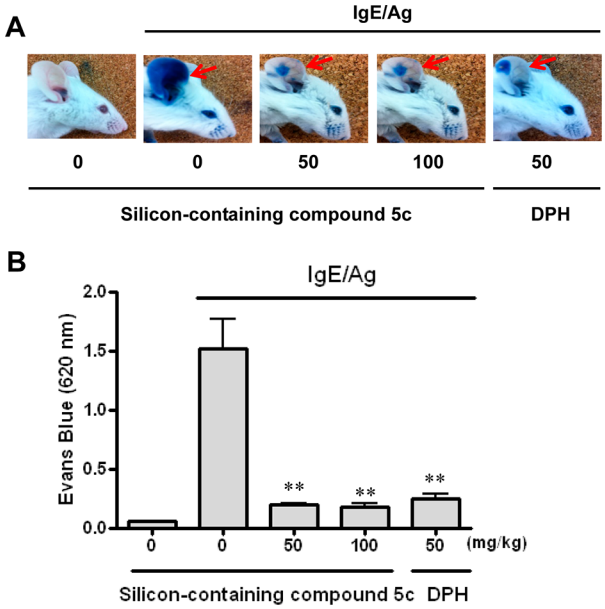

Figure 7. Effect of silicon-containing compound **5c** on IgE/Ag-induced passive cutaneous anaphylaxis (PCA). Seven-week-old ICR mice were intravenously injected with 200 μg of antigen containing 3% Evans blue 24 h after intradermal injection of DNP-IgE (0.5 μg) into the ear. Silicon-containing compound **5c** was orally administered 1 h before the antigen. (A) Representative pictures of the ears are shown. (B) The dye was extracted overnight in 500 μL of formamide at 63 °C, and the light absorbance was measured at 620 nm. Data are expressed as the mean ± SD (n = 4). The asterisks indicate significant difference from IgE/Ag-stimulated controls without silicon-containing compound **5c** ((**) $p < 0.001$). Diphenylhydramine (DPH, 50 mg/kg) was used as a typical anti-histamine reference drug.

was comparable to that of 50 mg/kg diphenylhydramine (DPH), an anti-histamine drug. These results confirm the ability of **5c** to inhibit passive cutaneous anaphylaxis in an in vivo animal model, similar to that in in vitro experiments.

In conclusion, we have demonstrated antiallergy activity of new and nontoxic sila-substituted 1,3,4-oxadiazoles using RBL-2H3 as the in vitro and in vivo models. We observed that 2-(4-chlorophenyl)-5-(((trimethylsilyl)methylsulfonyl)methyl)-1,3,4-oxadiazole (**5c**) effectively suppressed DNP-HSA-induced mast cell degranulation in a dose-dependent manner. Also, silyl compound **5c** effectively suppressed DNP-HSA-induced mast cell degranulation, compared to carbon analogue **9**. In addition **5c** greatly suppressed the expression of TNF-α mRNA and Akt phosphorylation in IgE/Ag-stimulated RBL-2H3 cells of the in vitro model. Furthermore we tested the effect of **5c** on passive cutaneous anaphylaxis in an in vivo PCA mouse model. When we fed **5c** orally to the IgE/Ag-sensitized, DNP-HSA-Evans blue dye injected mouse, the intensity of the blue dye decreased with increasing **5c** concentration, and **5c** also suppressed anaphylaxis in the mice. The suppression by **5c** was more effective than that by DPH, an anti-histamine drug. With all the experimental results, we believe that **5c** will be a useful clinical candidate for antiallergic drugs.

EXPERIMENTAL SECTION

Chemistry. Melting points were determined in open capillaries on a Stuart apparatus and are uncorrected. Reagents and solvents were obtained in the highest available purity and used without further purification unless otherwise indicated. The purity of the compounds was checked by TLC (silica gel, ethyl acetate/hexane (1:3)). The ^{1}H NMR, ^{13}C NMR, and ^{29}Si NMR spectra were recorded on a Bruker AMX 400 NMR spectrometer in $CDCl_3$. Mass spectra were recorded on a low-resolution (Agilent Technologies GC/MS model 6890N and model 5973N mass selective detector) EI mass spectrometer and a high-resolution (JEOL JMS-600W/JEOL JMS-700) instrument. The purities of the tested compounds, which were examined by GC/MS, were more than 95%.

2-((Trimethylsilyl)methylthio)acetic Acid (2). 2-((Trimethylsilyl)methylthio)acetic acid was prepared according to the literature procedure.[22] Condensation of (chloromethyl)trimethylsilane (**1**) (6.1 g, 50 mmol) with mercaptoacetic acid (4.6 g, 50 mmol) afforded the corresponding 2-((trimethylsilyl)methylthio)acetic acid (**2**) in 93% yield. A colorless liquid with a boiling point of 163−165 °C (760 Torr) was obtained. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.07 (s, 9H, Si-CH_3), 1.93 (s, 2H, Si-CH_2), 3.20 (s, 2H, S-CH_2), 9.61 (bs, 1H, OH). ^{13}C NMR ($CDCl_3$, 100 MHz): δ −1.81 (Si-CH_3), 19.44 (Si-CH_2), 37.54 (S-CH_2), 177.08 (C═O). ^{29}Si NMR ($CDCl_3$, 79 MHz): δ 1.34.

2-((Trimethylsilyl)methylsulfonyl)acetic Acid (3). 2-((Trimethylsilyl)methylsulfonyl)acetic acid was prepared according to the literature procedure.[22] Oxidation of 2-((trimethylsilyl)methylthio)-acetic acid (**2**) (2.7 g, 15 mmol) with 30% hydrogen peroxide (6.7 mL, 66 mmol) gave 2-((trimethylsilyl)methylsulfonyl)acetic acid (**3**) in 84% yield. A white solid with a melting point of 61−63 °C was obtained. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.28 (s, 9H, Si-CH_3), 2.97 (s, 2H, Si-CH_2), 4.03 (s, 2H, SO_2-CH_2), 9.65 (bs, 1H, OH). ^{13}C NMR ($CDCl_3$, 100 MHz): δ −0.65 (Si-CH_3), 44.82 (Si-CH_2), 61.19 (SO_2-CH_2), 167.38 (C═O). ^{29}Si NMR ($CDCl_3$, 79 MHz): δ 1.74.

General Procedure for the Preparation of 2-Aryl-5-(((trimethylsilyl)methylthio)-methyl)-1,3,4-oxadiazole (4a−c). A mixture of 2-((trimethylsilyl)methylthio)acetic acid (**2**) (1.8 g, 10 mmol), arylhydrazide (10 mmol), and $POCl_3$ (7.0 mL, 75 mmol) was heated at about 70 °C for 4−6 h. The excess $POCl_3$[23] was removed under reduced pressure, and the residue was poured into crushed ice. The resulting precipitate was filtered, washed with saturated sodium bicarbonate solution and then with water, dried, and recrystallized from ethanol to afford **4a−c**.

2-Phenyl-5-(((trimethylsilyl)methylthio)methyl)-1,3,4-oxadiazole (4a). Compound **4a** was obtained from 2-((trimethylsilyl)-methylthio)acetic acid) (**2**) (1.8 g, 10 mmol), benzohydrazide (1.4 g, 10 mmol), and $POCl_3$ (7.0 mL, 75 mmol) using the general procedure for **4a−c**. A pale yellow solid with a melting point of 82−84 °C was obtained in 91% yield. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.05 (s, 9H, Si-CH_3), 1.89 (s, 2H, Si-CH_2), 3.87 (s, 2H, S-CH_2), 7.49−8.05 (m, 5H, Ph). ^{13}C NMR ($CDCl_3$, 100 MHz): δ −1.77 (Si-CH_3), 18.76 (Si-CH_2), 29.16 (S-CH_2), 123.83, 126.93, 129.06, 131.78 (Ph), 164.04 (C-2), 165.38 (C-5). ^{29}Si NMR ($CDCl_3$, 79 MHz): δ 1.52. MS: m/z (relative intensity) 263 (M^+ − 15, 24.2), 231 (100), 160 (57.0), 105 (23.4), 100 (14.8), 73 (46.8). HRMS: $C_{13}H_{19}N_2OSSi$ 279.0987 (M^+ + 1, calcd), 279.0992 (found).

2-*p*-Tolyl-5-(((trimethylsilyl)methylthio)methyl)-1,3,4-oxadiazole (4b). Compound **4b** was obtained from 2-((trimethylsilyl)-methylthio)acetic acid) (**2**) (1.8 g, 10 mmol), 4-Me-benzohydrazide

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(1.5 g, 10 mmol), and $POCl_3$ (7.0 mL, 75 mmol) using the general procedure for **4a–c**. A white solid with a melting point of 89–91 °C was obtained in 89% yield. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.04 (s, 9H, Si-CH_3), 1.88 (s, 2H, Si-CH_2), 2.39 (s, 3H, Ph-CH_3), 3.85 (s, 2H, S-CH_2), 7.27 (d, 2H, Ph), 7.91 (d, 2H, Ph). ^{13}C NMR ($CDCl_3$, 100 MHz): δ −1.79 (Si-CH_3), 18.71 (Si-CH_2), 21.61 (Ph-CH_3), 29.11 (S-CH_2), 121.04, 126.85, 129.72, 142.27 (Ph), 163.74 (C-2), 165.46 (C-5). ^{29}Si NMR ($CDCl_3$, 79 MHz): δ 1.45. MS: *m*/*z* (relative intensity) 277 (M^+ − 15, 24.2), 245 (100), 174 (63.2), 119 (54.4), 100 (16.1), 91 (41.1), 73 (82.3). HRMS: $C_{14}H_{21}N_2OSSi$ 293.1144 (M^+ + 1, calcd), 293.1134 (found).

2-(4-Chlorophenyl)-5-(((trimethylsilyl)methylthio)methyl)-1,3,4-oxadiazole (4c). Compound **4c** was obtained from 2-((trimethylsilyl)methylthio)acetic acid) (**2**) (1.8 g, 10 mmol), 4-Cl-benzohydrazide (1.7 g, 10 mmol), and $POCl_3$ (7.0 mL, 75 mmol) using the general procedure for **4a–c**. A white solid with a melting point of 97–99 °C was obtained in 93% yield. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.05 (s, 9H, Si-CH_3), 1.88 (s, 2H, Si-CH_2), 3.86 (s, 2H, SO_2-CH_2), 7.46 (d, 2H, Ph), 7.97 (d, 2H, Ph). ^{13}C NMR ($CDCl_3$, 100 MHz): −1.78 (Si-CH_3), 18.80 (Si-CH_2), 29.14 (S-CH_2), 122.29, 128.20, 129.46, 138.07 (Ph), 164.20 (C-2), 164.58(C-5). ^{29}Si NMR ($CDCl_3$, 79 MHz): δ 1.54. MS: *m*/*z* (relative intensity) 297 (M^+ − 15, 25.0), 265 (100), 194 (63.2), 139 (50.7), 111 (30.4), 100 (26.5), 73 (89.0). HRMS: $C_{13}H_{18}ClN_2OSSi$ 313.0598 (M^+ + 1, calcd), 313.0598 (found).

General Procedure for the Synthesis of 2-Aryl-5-(((trimethylsilyl)methylsulfonyl)methyl)-1,3,4-oxadiazole (5a–c). A mixture of arylhydrazide (10 mmol), 2-((trimethylsilyl)-methylsulfonyl)acetic acid (**3**) (2.1 g, 10 mmol), and $POCl_3$ (7.0 mL, 75 mmol) was heated at about 70 °C for 4–6 h. The excess $POCl_3$ was removed under reduced pressure, and the residue was poured into crushed ice. The resulting precipitate was filtered, washed with saturated sodium bicarbonate solution and then with water, dried, and recrystallized from ethanol to give **5a–c**.

2-Phenyl-5-(((trimethylsilyl)methylsulfonyl)methyl)-1,3,4-oxadiazole (5a). Compound **5a** was obtained from 2-((trimethylsilyl)methylsulfonyl)acetic acid (**3**) (2.1 g, 10 mmol), benzohydrazide (1.4 g, 10 mmol), and $POCl_3$ (7.0 mL, 75 mmol) using the general procedure for **5a–c**. A white solid with a melting point of 102–104 °C was obtained in 89% yield. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.26 (s, 9H, Si-CH_3), 2.90 (s, 2H, Si-CH_2), 4.56 (s, 2H, SO_2-CH_2) 7.50–8.07 (m, 5H, Ph). ^{13}C NMR ($CDCl_3$, 100 MHz): δ −0.67 (Si-CH_3), 43.35 (Si-CH_2), 53.45 (SO_2-CH_2), 123.15, 127.20, 129.18, 132.33 (Ph), 157.96 (C-2), 166.45(C-5). ^{29}Si NMR ($CDCl_3$, 79 MHz): δ 2.05. MS: *m*/*z* (relative intensity) 310 (M^+, 9.6), 296 (8.3), 231 (20.1), 159 (89.5), 105 (100), 77 (60.4). HRMS: $C_{13}H_{18}N_2O_3SSi$ 310.0807 (M^+, calcd), 310.0807 (found).

2-*p*-Tolyl-5-(((trimethylsilyl)methylsulfonyl)methyl)-1,3,4-oxadiazole (5b). Compound **5b** was obtained from 2-((trimethylsilyl)methylsulfonyl)acetic acid (**3**) (2.1 g, 10 mmol), 4-Me-benzohydrazide (1.5 g, 10 mmol), and $POCl_3$ (7.0 mL, 75 mmol) using the general procedure for **5a–c**. A white solid with a melting point of 117–119 °C was obtained in 86% yield. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.25 (s, 9H, Si-CH_3), 2.40 (s, 3H, Ph-CH_3), 2.89 (s, 2H, Si-CH_2), 4.55 (s, 2H, SO_2-CH_2), 7.29 (d, 2H, Ph), 7.92 (d, 2H, Ph). ^{13}C NMR ($CDCl_3$, 100 MHz): δ −0.68 (Si-CH_3), 21.68 (Ph-CH_3), 43.37 (Si-CH_2), 53.44 (SO_2−CH_2), 120.36, 127.14, 129.86, 142.99 (Ph), 157.67 (C-2), 166.58(C-5). ^{29}Si NMR ($CDCl_3$, 79 MHz): δ 1.98. MS: *m*/*z* (relative intensity) 324 (M^+, 7.2), 309 (10.9), 245 (5.1), 173 (75.9), 119 (100), 91 (31.5), 73 (24.8). HRMS: $C_{14}H_{20}N_2O_3SSi$ 324.0964 (calcd), 324.0961 (found).

2-(4-Chlorophenyl)-5-(((trimethylsilyl)methylsulfonyl)-methyl)-1,3,4-oxadiazole (5c). Compound **5c** was obtained from 2-((trimethylsilyl)methylsulfonyl)acetic acid (**3**) (2.1 g, 10 mmol), 4-Cl-benzohydrazide (1.7 g, 10 mmol), and $POCl_3$ (7.0 mL 75 mmol) using the general procedure for **5a–c**. A white solid with a melting point of 126–128 °C was obtained in 91% yield. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.26 (s, 9H, Si-CH_3), 2.89 (s, 2H, Si-CH_2), 4.56 (s, 2H, SO_2-CH_2), 7.48 (d, 2H, Ph), 8.00 (d, 2H, Ph). ^{13}C NMR ($CDCl_3$, 100 MHz): δ −0.67 (Si-CH_3), 43.40 (Si-CH_2), 53.40 (SO_2-CH_2), 121.60, 128.47, 129.61, 138.73 (Ph), 158.11 (C-2), 165.66 (C-5). ^{29}Si NMR ($CDCl_3$, 79 MHz): δ 2.11. MS: *m*/*z* (relative intensity) 344 (M^+, 9.1), 329 (13.7), 267 (8.6), 193 (85.4), 139 (100), 111 (22.5), 73 (17.7). HRMS: $C_{13}H_{17}ClN_2O_3SSi$ 344.0418 (M^+, calcd), 344.0423 (found).

2-(Neopentylthio)acetic Acid (7). To a solution of potassium hydroxide (2.8 g, 50 mmol) in ethanol (30 mL) was slowly added mercaptoacetic acid (2.3 g, 25 mmol), and then neopentyl bromide (3.8 g, 25 mmol) was added portionwise. The mixture was refluxed for 8 h, was cooled, and was poured into ice-cold water containing hydrochloric acid. The resultant liquid was extracted with diethyl ether and was distilled to afford 2-(neopentylthio)acetic acid (**7**) in 95% yield. A colorless liquid with a boiling point of 118–120 °C (760 Torr) was obtained. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 0.91 (s, 9H, C-CH_3), 2.52 (s, 2H, C-CH_2), 3.17 (s, 2H, S-CH_2), 11.69 (bs, 1H, OH). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 28.76 (-CH_3), 32.26 (*C*-CH_3) 41.32 (C-CH_2), 47.70 (S-CH_2), 177.36 (C=O). MS: *m*/*z* (relative intensity) 162 (M^+, 97.0), 129 (11.1), 106 (97.0), 101 (39.5), 77 (14.9), 71 (18.6), 57 (100).

2-(Neopentylsulfonyl)acetic Acid (8). 2-(Neopentylsulfonyl)-acetic acid was prepared according to the literature procedure.[22] Oxidation of 2-(neopentylthio)acetic acid (**7**) (2.4 g, 15 mmol) with 30% hydrogen peroxide (6.0 mL, 59 mmol) gave 2-(neopentylsulfonyl)acetic acid (**8**) in 81% yield. A colorless liquid with a boiling point of 134–136 °C (760 Torr) was obtained.^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.17 (s, 9H, C−CH_3), 3.23 (s, 2H, C-CH_2), 3.99 (s, 2H, SO_2-CH_2), 9.89 (bs, 1H, OH). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 29.71 (CH_3), 32.37 (*C*-CH_3), 60.29 (C-CH_2) 64.50 (SO_2-CH_2), 177.84 (C=O).

2-(4-Chlorophenyl)-5-(neopentylsulfonylmethyl)-1,3,4-oxadiazole (9). 2-(4-Chlorophenyl)-5-(neopentylsulfonylmethyl)-1,3,4-oxadiazole (**9**) was prepared according to the literature procedure.[23] A mixture of 4-Cl-benzohydrazide (1.7 g, 10 mmol), 2-(neopentylsulfonyl)acetic acid (**8**) (2.0 g, 10 mmol), and $POCl_3$ (7.0 mL, 75 mmol) was heated at about 70 °C for 5 h to give **9**. A white solid with a melting point of 102–104 °C was obtained in 79% yield. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.19 (s, 9H, C-CH_3), 3.20 (s, 2H, C-CH_2), 4.56 (s, 2H, SO_2-CH_2), 7.48 (d, 2H, Ph), 7.98 (d, 2H, Ph). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 29.69 (CH_3), 32.45 (*C*-CH_3), 52.36 (C−CH_2), 63.49 (SO_2-CH_2), 121.45, 128.49, 129.63, 138.84 (Ph), 157.71 (C-2), 165.73(C-5). MS: *m*/*z* (relative intensity) 328 (M^+, 5.1), 313 (5.1), 193 (63.7), 139 (100), 123 (7.4), 111 (36.2), 75 (14.8), 55 (22.9). HRMS: $C_{14}H_{17}ClN_2O_3S$ 328.0648 (M^+, calcd), 328.0650 (found).

Materials. Mouse monoclonal anti-dinitrophenol (anti-DNP) IgE antibody, dinitrophenol-conjugated human serum albumin (DNP-HSA), and Evans blue were obtained from Sigma-Aldrich, (St. Louis, MO, U.S.). *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide was obtained from MP Biomedicals, LLC (Solon, OH, U.S.).

Cell Culture. The rat mast cell line RBL-2H3 was obtained from the American Type Culture Collection (ATCC, Rockville, MD, U.S.). The cells were grown in Eagle's minimal essential medium (WelGENE, Inc., Daegu, Korea) containing 10% (v/v) fetal bovine serum and 100 units/mL penicillin–streptomycin (Lonza Walkersville, Inc., Walkersville, MD, U.S.) at 37 °C in a humidified 5% CO_2 atmosphere. Cells were detached with trypsin-EDTA (ethylenediaminetetraacetic acid) solution, washed with phosphate buffered saline (PBS, pH 7.2), and resuspended in fresh medium for use in subsequent experiments.

Measurement of β-Hexosaminidase Release. We measured the release of β-hexosaminidase as a marker for degranulation. RBL-2H3 cells were distributed in 24-well plates (2×10^5 cells/well) and were sensitized overnight with anti-DNP-specific IgE at 200 ng/mL. The IgE-sensitized cells were washed twice with PIPES buffer (25 mM PIPES, pH 7.2, 110 mM NaCl, 4 mM KCl, 0.4 mM MgCl, 40 mM HCl, 5.6 mM glucose, 1 mM $CaCl_2$, and 0.1% BSA). They were then treated for 30 min at 37 °C with the silicon-containing compounds in PIPES buffer at the indicated concentrations. The cells were subsequently stimulated with DNP-HSA (25 ng/mL) at 37 °C for 15 min and chilled on ice to stop the stimulation. To measure the β-hexosaminidase release, the supernatant from the antigen (Ag)

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stimulated cells in PIPES buffer and β-hexosaminidase substrate (*p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide) in 0.1 M citrate buffer (pH 4.5) were mixed in 96-well plates and incubated at 37 °C for 1 h. This reaction was terminated by adding 0.1 M carbonate buffer (pH 10.5), and the absorbance was measured at 405 nm using a microplate reader.

Measurement of Cell Viability. The Ez-cytox enhanced cell viability kit (Daeil Labservice, Seoul, Korea) was used to assess cell viability following the manufacturer's instructions. In brief, RBL-2H3 cells (2×10^4 cells/well) were seeded into 96-well plates and incubated overnight with 200 ng/mL anti-DNP-specific IgE. After incubation, the medium was replaced with serum free medium, and the cells were treated with silicon-containing compound **5c** and 25 ng/mL DNP-HSA for 23 h. The Ez-cytox kit reagent was then added to the medium, and the cells were incubated at 37 °C for 1 h. Cell viability was determined based on absorbance at 450 nm measured with a microplate reader.

Animals. Seven-week-old male ICR mice were purchased from Orient Bio (Gangneung, Korea) and housed in wire cages at 20−22 °C and a relative humidity of 40−50%. All animals were given access to standard laboratory chow and water ad libitum. The Institutional Animal Care and Use Committee (IACUC) at Yonsei University (Wonju, Korea) approved the protocol for this study.

Induction of Mast-Cell-Mediated Passive Cutaneous Anaphylaxis in Mice. An anti-DNP-specific IgE antibody (0.5 μg) was injected intradermally into one ear of each mouse. After 24 h, the mice were challenged with an intravenous injection of DNP-HSA (200 μg; antigen, Ag) in 200 μL of PBS 3% Evans blue. One hour later, the silicon-containing compound **5c** was administered orally at a dose of 50−100 mg/kg. Two hours after the Ag challenge, the mice were euthanized and the treated ear was excised to measure the amount of dye that had extravasated in response to the Ag. The dye was extracted from the ear in 500 μL of formamide at 63 °C overnight and quantified by measuring dye absorbance at 620 nm.

RNA Extraction and Reverse Transcription Polymerase Chain Reaction (RT-PCR). The RBL-2H3 cells (8×10^5 cells/well) were seeded into six-well plates and incubated overnight in a medium containing 200 ng/mL anti-DNP-specific IgE. The cells were washed twice, resuspended in PIPES buffer, and stimulated with DNP-HSA (25 ng/mL) for 1 h with and without silicon-containing compound **5c**. After incubation, the cells were washed twice with ice-cold PBS. Total RNA was isolated using TRI reagent (Sigma-Aldrich, St. Louis, MO, U.S.) according to the manufacturer's instructions. The concentration of total RNA was determined using a spectrophotometer. The total RNA (1 μg) was used as the template for cDNA synthesis and PCR using the Accupower RT/PCR premix kit (Bioneer, Daejeon, Korea). The following primers were used: rat TNF-α sense 5′-CAAGGAGGAGAAGTTCCCAA-3′; TNF-α antisense 5′-CGGACTCCGTGATGTCTAAG-3′; β-actin sense 5′-ATGCCATCCTGCGTCTGGACCTGGC-3′; β-actin antisense 5′-AGCATTTGCGGTGCACGATGGAGGG-3′. The denaturation, annealing, extension conditions, and number of cycles were 94 °C for 60 s, 49 °C for 45 s, 72 °C for 45 s, and 35 cycles, respectively, for TNF-α. The PCR products were electrophoresed on a 2% agarose gel and visualized by adding ethidium bromide. The gels were examined using a transilluminator (Vilber Lourmat, France).

Protein Extraction and Western Blotting Analysis. To extract protein for Western blot analysis, RBL2H3 cells (8×10^5 cells/ml) were seeded in a six-well plate for 24 h at 37 °C with 5% CO_2. The medium was removed and replaced with fresh medium containing various concentrations (0−200 μM) of silicon-containing compound **5c** and stimulated with DNP-HSA (25 ng/mL) for 1 h. The cells were then washed twice with ice-cold PBS (pH 7.2) and collected on ice. The washed cell pellets were centrifuged at 12000*g* for 10 min at 4 °C. The supernatant was removed from the tubes, and 150 μL of PROPREP (iNtRON Biotechnology, Inc., Seongnam, Korea) was added to each tube. The cells were placed on ice for 1 h and sonicated to decrease viscosity. Insoluble cell debris was removed by centrifugation at 12000*g* for 10 min at 4 °C. The supernatants were collected, and the protein concentration was measured using the Bradford assay (Bio-Rad, CA, U.S.) according to the manufacturer's instruction. Total cellular proteins (20 μg) were separated by 10% SDS−polyacrylamide gel electrophoresis and transferred to a polyvinylidene fluoride (PVDF) membrane (Bio-Rad, CA, U.S.) using a Trans-Blot SD semidry transfer cell (Bio-Rad, CA, U.S.). Membranes were incubated for 1 h with blocking solution (5% skim milk) at room temperature, followed by incubation overnight at 4 °C with specific primary antibodies (1:2000). Subsequently, membranes were washed three times with TBS-T buffer and incubated with a secondary anti-rabbit antibody (1:5000) for 1 h at room temperature. The membranes were then washed three times with TBS-T, and the proteins were detected using an enhanced chemiluminescence Western blotting detection kit (GE Healthcare, U.K.).

Statistical Analysis. Experimental results are expressed as the mean ± SD. A one-way analysis of variance (ANOVA) with the Dunnett's test for multiple comparisons was performed. $p < 0.01$ and $p < 0.001$ were considered statistically significant, as indicated.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Spectra for compounds **4**−**9**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

§The first two authors contributed equally to this work.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

RBL-2H3, rat basophilic leukemia; PCA, passive cutaneous anaphylaxis; DNP-HSA, dinitrophenylated human serum albumin; FcεR1, high-affinity IgE receptor; TNF-α, tumor necrosis factor α; mRNA, messenger ribonucleic acid; DPH, diphenylhydramine; IgE, immunoglobulin E; RT-PCR, reverse transcription polymerase chain reaction; IACUC, Institutional Animal Care and Use Committee; Ag, antigen

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