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Structure−Activity Relationship Study on Isothiocyanates: Comparison of TRPA1-Activating Ability between Allyl Isothiocyanate and Specific Flavor Components of Wasabi, Horseradish, and White Mustard

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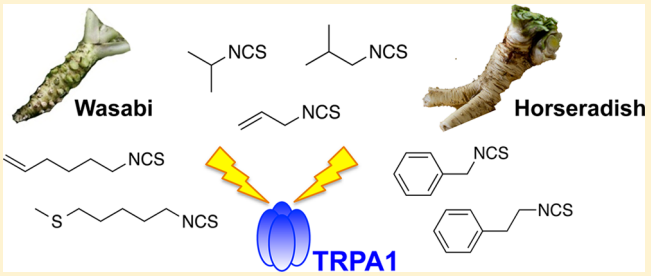
Supporting Information

ABSTRACT: Allyl isothiocyanate (ITC) (**4**) is the main pungent component in wasabi, and it generates an acrid sensation by activating TRPA1. The flavor and pungency of ITCs vary depending on the compound. However, the differences in activity to activate TRPA1 between ITCs are not known except for a few compounds. To investigate the effect of carbon chain length and substituents of ITCs, the TRPA1-activiting ability of 16 ITCs was measured. Since most of the ITCs showed nearly equal TRPA1-activiting potency, the ITC moiety is likely the predominant contributor to their TRPA1-activating abilities, and contributions of other functional groups to their activities to activate TRPA1 are comparatively small.



Wasabi (*Wasabia japonica* Matsumura), horseradish (*Cochlearia armoracia* L.), and white mustard (*Sinapis alba* L.) belong to the *Cruciferae* family. When tissues of these plants are damaged, isothiocyanates (ITCs) are liberated from their precursors, glucosinolates.[1] The characteristic flavors of grated wasabi, horseradish, and white mustard are attributed to the ITC components.

Allyl ITC (**4**) is the main spicy component of wasabi, horseradish, and white mustard. It is highly pungent and elicits a lachrymatory sensation. Alkyl ITCs [isopropyl ITC (**1**), *sec*-butyl ITC (**2**), and isobutyl ITC (**3**)] are found in both grated wasabi and horseradish and have a chemical-like odor. *ω*-Alkenyl ITCs [3-butenyl ITC (**5**), 4-pentenyl ITC (**6**), 5-hexenyl ITC (**7**), and 6-heptenyl ITC (**8**)] are important for green notes of wasabi. *ω*-Methylthioalkyl ITCs [5-methylthiopentyl ITC (**14**), 6-methylthiohexyl ITC (**15**), and 7-methylthioheptyl ITC (**16**)] give wasabi a radish-like, sweetish smell. In particular, **15** is an important contributor to the radish-like odor of wasabi and to the richness in flavor. While **4** is a strong irritant, *ω*-alkenyl ITCs (**5**−**8**) and *ω*-methylthioalkyl ITCs (**14**−**16**) have milder flavors. Benzyl ITC (**9**) and phenylethyl ITC (**10**) are characteristic components of grated horseradish. Compound **9** has a chemical-like smell, and **10** possesses a strong radish-like flavor. Since the content of **10** in horseradish is relatively high, it is considered an important contributor to the radish-like flavor of horseradish. *p*-Hydroxybenzyl ITC (**11**), a component specific to white mustard, is characterized by its mild pungency.[2−6]

Compound **4** induces an acrid sensation by activating transient receptor potential ankyrin 1 (TRPA1).[7] TRPA1 is a nonselective cation channel and is mainly expressed in primary sensory neurons.[8] In the beginning, TRPA1 was identified as a cold receptor.[8] However, whether mammalian TRPA1 is a receptor for noxious cold is highly controversial, as channel activity by cold was observed by some groups but not by others.[9] Recently, it is reported that human TRPA1 is cold insensitive.[10] TRPA1 is activated by pungent compounds, such as cinnamaldehyde (derived from cinnamon), allicin (found in crushed garlic), and piperine (found in pepper).[8,11−13] The TRPA1-activating ability of **4**, **9**, **10**, and **15** has been reported.[14,15] Furthermore, it has been shown that **4** activates capsaicin (CAP) receptor TRP vanilloid (TRPV) 1 at a high concentration.[14,15] TRPA1 and menthol receptor TRP melastatin (TRPM8) have common agonists, such as menthol and icilin.[16] But it has been shown that **4** and **15** do not have an agonist activity on TRPM8.[11,15]

ITC flavors have been well analyzed, and their odors and pungencies depend on the ITC structure. However, the differences in a TRPA1-activating ability between ITC compounds are not clear. To investigate whether the carbon chain length, benzene ring, and substituents of ITCs affect their TRPA1-activating potencies, a chemical structure and a TRPA1-activiting ability relationship study was performed. In

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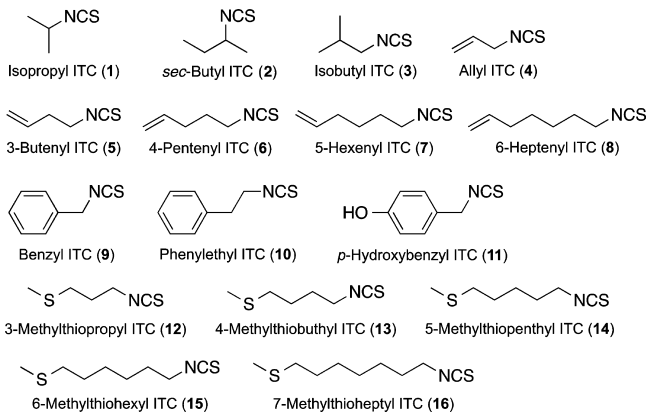
addition, activities to activate TRPV1 and TRPM8 of the ITCs were measured. In this study, 16 ITCs derived from wasabi, horseradish, and white mustard were examined.



Activation of TRPA1 by Compounds 1−16. A 1 μM concentration of compounds **1**−**10** and **12**−**16** and 30 μM *p*-hydroxybenzyl ITC (**11**) elicited Ca^{2+} influx on TRPA1-expressing HEK cells. These responses were significantly attenuated by cotreatment with TRPA1 antagonist HC-030031, and these compounds did not cause a Ca^{2+} response in TREx HEK cells, which do not express TRPA1 (Figure 1A). These results show that compounds **1**−**16** have a TRPA1 activation potency. Figure 1B shows the concentration−response curves of compounds **1**−**16**. Table 1 shows the EC_{50} value and maximum response (MAX) of each compound. Among the tested compounds, 14 of them (**1**−**3**, **5**−**10**, and **12**−**16**) showed a TRPA1 activation potency that was either stronger than or equal to that of allyl ITC (**4**) (EC_{50}: 0.58 μM, MAX: 97%). Benzyl ITC (**9**), the horseradish-specific volatile, showed the strongest activity to activate TRPA1 (EC_{50}: 0.25 μM, MAX: 95%). Its EC_{50} value was approximately half that of **4**, and its maximum response was almost equal to that of **4**. *p*-Hydroxybenzyl ITC (**11**), a characteristic component of white mustard, had the lowest TRPA1-activating ability among the compounds tested (EC_{50}: 20 μM, MAX: 96%). Its EC_{50} value was 40 times higher than that of **4**, and its TRPA1-activating potency was quite weak compared to those of other ITCs. Among ITCs with similar functional groups, it was found that the TRPA1-activating ability decreased slightly as the carbon chain length increased. However, since the TRPA1 activation potencies of most ITCs were nearly equal, their ITC moieties are likely the predominant contributors to their TRPA1-activating abilities, and contributions of other structure features (carbon chain length, thiol moiety, and double bond) to their TRPA1 activation potencies are comparatively small.

Activity to Activate TRPV1 of Compounds 1−16. Compounds **1**−**16** were each administered at 300 μM to HEK cells stably expressing TRPV1. Six of them, two alkyl ITCs (**1** and **2**), allyl ITC (**4**), and aryl ITCs (**9**−**11**), elicited relatively strong responses in TRPV1-expressing cells. Responses to the ω-alkenyl ITCs (**5**−**8**) and ω-methylthioalkyl ITCs (**12**−**16**) were quite weak (Figure 2A). The Ca^{2+} influx induced by the six aforementioned ITCs (**1**, **2**, **4**, **9**−**11**) was significantly inhibited by addition of TRPV1 antagonist capsazepine (CPZ), and the responses in TREx HEK cells were significantly weaker than those in TRPV1-expressing HEK cells (Figure 2B). These results indicated that these six ITCs activate TRPV1. Figure 2C shows the concentration−response curves of these compounds.

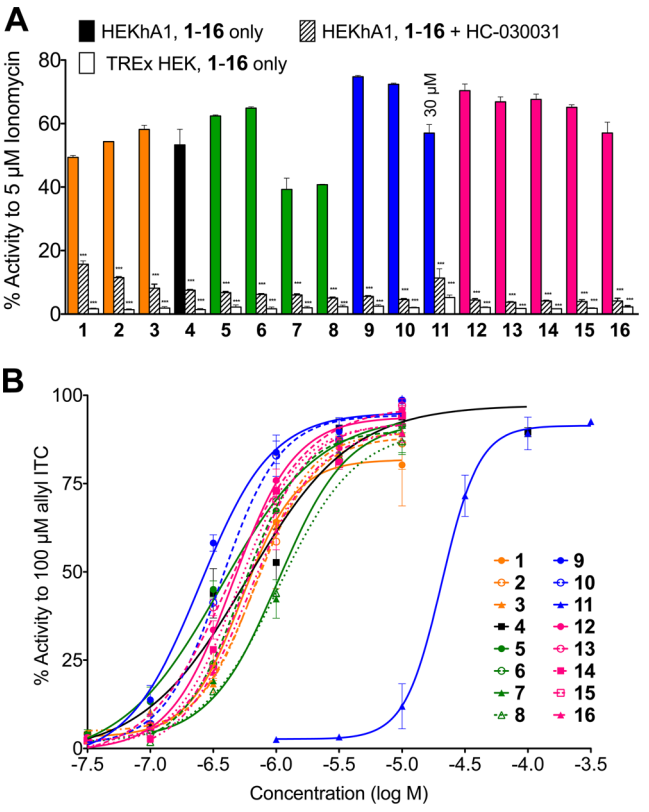


Figure 1. TRPA1-activating potency of compounds **1**−**16**. Each ITC group is colored as follows: alkyl ITCs, orange; allyl ITC, black; ω-alkenyl ITCs, green; aromatic ITCs, blue; ω-methylthioalkyl ITCs, pink. (A) 1 μM compounds **1**−**10** and **12**−**16** and 30 μM **11** were administered to TRPA1-expressing HEK cells. *y*-Axis: relative activity to 5 μM ionomycin (%). Solid column, Ca^{2+} response by ITCs in TRPA1-expressing cells; slant-lined column, TRPA1 antagonist HC-030031 was cotreated with compounds **1**−**16** and administered to TRPA1-expressing cells; unfilled column, compounds **1**−**16** were added to TREx HEK cells not expressing TRPA1. (B) Concentration−response curves of **1**−**16**. *x*-Axis: agonist concentration (log M); *y*-axis: relative activity to 100 μM **4**. Each data point represents the mean ± SEM, n = 3−8 [*** indicates $p < 0.0005$ (unpaired t-test)].

Compounds **1** (EC_{50}: 100 μM, MAX: 50%) and **2** (EC_{50}: 100 μM, MAX: 62%) had slightly lower EC_{50} values and maximum responses that were 2 to 3 times larger than those of **4** (EC_{50}: 160 μM, MAX: 25%). The aromatic ITCs, **9** (EC_{50}: 223 μM, MAX: 49%), **10** (EC_{50}: 180 μM, MAX: 43%), and **11** (EC_{50}: 170 μM, MAX: 63%), showed slightly higher EC_{50} values than did **4**, but their maximum responses were 2 to 3 times larger.

Agonist Activity of Compounds 1−16 on TRPM8. Compounds **1**−**16** (300 μM) were each used to treat HEK cells stably expressing TRPM8. As a result, all compounds did not elicit any Ca^{2+} responses (data not shown). It has been reported that **4** and **15** do not activate TRPM8, and our result was consistent with the previous reports.[11,15]

Except for **11** (EC_{50}: 20 μM), all ITCs had high TRPA1 activation potencies (EC_{50}: 0.25−1 μM; MAX: 80−95%). Regarding their TRPV1-activating abilities, most of them did not evoke a response in TRPV1-expressing cells, and only six of them (**1**, **2**, **4**, and **9**−**11**) weakly activated TRPV1. Their EC_{50} values were between 100 and 200 μM, and they had low maximum responses. Accordingly, the ITCs activate TRPA1 much more strongly than they activate TRPV1. Although the odors and irritancies of the ITCs vary depending on the

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Table 1. TRPA1 and TRPV1-Activating Abilities of Compounds 1−16

compound name	TRPA1 activation potency		TRPV1 activation potency	
	EC_{50} (μM)	Top (%)[a]	EC_{50} (μM)	Top (%)[b]
isopropyl ITC (**1**)	0.53	82	100	50
sec-butyl ITC (**2**)	0.68	88	100	62
isobutyl ITC (**3**)	0.69	89	–[c]	
allyl ITC (**4**)	0.58	97	160	25
3-butenyl ITC (**5**)	0.36	95	–	
4-pentenyl ITC (**6**)	0.54	90	–	
5-hexenyl ITC (**7**)	1.04	93	–	
6-heptenyl ITC (**8**)	1.05	90	–	
benzyl ITC (**9**)	0.25	95	223	49
phenylethyl ITC (**10**)	0.37	94	180	43
p-hydroxybenzyl ITC (**11**)	20	96	170	63
3-methylthiopropyl ITC (**12**)	0.45	94	–	
4-methylthiobutyl ITC (**13**)	0.42	97	–	
5-methylthiopentyl ITC (**14**)	0.5	92	–	
6-methylthiohexyl ITC (**15**)	0.58	92	–	
7-methylthioheptyl ITC (**16**)	0.61	90	–	

[a]Percent response to 100 μM allyl ITC (**4**). [b]Percent response to 10 μM CAP (capsaicin). [c]– indicates no TRP-activating ability.

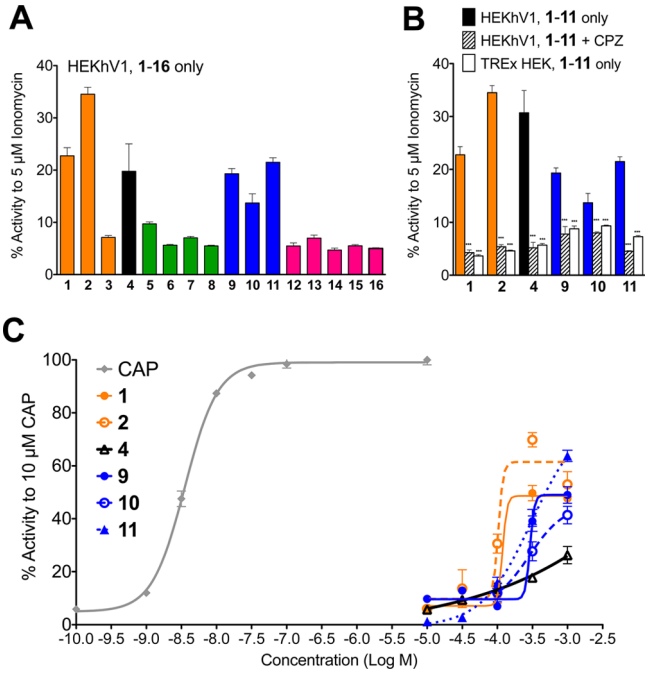

Figure 2. TRPV1-activating ability of compounds **1**−**16**. Each ITC group and positive controls were classified with the following different colors: alkyl ITCs, orange; allyl ITC, black; ω-alkenyl ITCs, green; aromatic ITCs:, blue; ω-methylthioalkyl ITCs, pink; capsaicin (CAP), gray. (A) Ca^{2+} influx elicited by 300 μM **1**−**16** on TRPV1-expressing HEK cells. (B) Solid column: responses by 300 μM **1**, **2**, **4**, and **9**−**11** in TRPV1-expressing cells; lined column: TRPV1 antagonist capsazepine (CPZ); each ITC compound was coadded to TRPV1-expressing cells; unfilled column: responses by **1**, **2**, **4**, and **9**−**11** in TREx HEK cells not expressing TRPV1. (C) Concentration−response curves of **1**, **2**, **4**, and **9**−**11**. *x*-Axis: agonist concentration (log M); *y*-axis: relative activity to 10 μM CAP. Data are expressed as percent responses to the ionophore ionomycin (5 μM; A and B) and 10 μM CAP (C). Each data point represents the mean ± SEM, n = 3−7 [*** means $p < 0.0005$ (unpaired t-test)].

structures of the compounds, most of them showed nearly equal activity to activate TRPA1. Therefore, the ITC moiety is considered the most important factor for their TRPA1-activating potencies, and the effects of their other functional groups (carbon chain length, double bond, benzene ring, and thiol moiety) against TRPA1-activating abilities are small in comparison.

Alkyl ITCs (**1** and **2**) are found in both wasabi and horseradish.[3,5] Their activities to activate TRPA1 were almost equal to that of **4**, and their TRPV1 activation potencies were stronger than that of **4**. ω-Alkenyl ITCs (**5**−**8**) and ω-methylthioalkyl ITCs (**12**−**16**) are wasabi-specific components and showed the same ability to activate TRPA1 as did **4**, but did not elicit TRPV1 activation. Benzyl ITC (**9**) and **10** are horseradish-specific volatiles, and their EC_{50} values on TRPA1 are half that of **4**. In terms of activity to activate TRPV1, these ITCs had almost equal activation potencies to that of **4**.

We compared the contents of each ITC compound with that of **4** in wasabi, and their contents were 100 times lower than that of **4**.[2] Accordingly, the main TRPA1 agonist of wasabi is **4**. In horseradish, the content of **10** is relatively high and is one-fifth that of **4**.[4] The TRPA1 activation potency of **10** was slightly higher than that of **4**; therefore, it was speculated that **10** has some contribution to the activation of TRPA1 by horseradish.

The TRPA1 activation mechanism of **4** has been reported. Its ITC moiety covalently binds to a cysteine residue located in the ankyrin repeat in the cytosolic N-terminus of TRPA1 and triggers TRPA1 activation.[17,18] It has also been shown that **15** activates TRPA1 in the same manner that **4** does.[15] Accordingly, ITC compounds used in this study are speculated to activate TRPA1 through the same mechanism that **4** does.

It has been reported that **4** elicits a strong acrid sensation, while ω-alkenyl ITCs (**5**−**8**) and ω-methylthioalkyl ITCs (**12**−**16**) have milder flavors.[2] Although they have low pungency, this study and a study by Uchida et al.[15] showed that they activate TRPA1 to the same extent that **4** does. TRPA1 is also activated by an alkaline pH, and cornea and nasal mucosa pains induced by ammonia vapors are considered the result of TRPA1 activation expressed in the trigeminal nerve. Since **4** is highly volatile and causes a painful sensation similar to that caused by vaporized ammonia, it is thought that **4** induces a noxious stimulus in the same way that ammonia does; **4** evaporates to the nasal cavity from the oral cavity and generates a lachrymatory sensation by activating TRPA1 expressed in the trigeminal nerve innervating the nasal cavity.[19] Accordingly, the volatility of the ITC compounds may be directly related, important for generating their irritancy. Since vapor pressure is an index for the volatility of chemical compounds, we compared the vapor pressure of **4** with those of the flavor components of wasabi (**7**, **8**, **15**, and **16**). The vapor pressure values, quoted from SciFinder, of **7**, **8**, **15**, **16**, and **4** were 0.25, 0.09, 0.005, 0.002, and 4.57 mmHg, respectively. A higher vapor pressure corresponds to higher volatility, and the vapor pressures of compounds **7**, **8**, **15**, and **16** were 100 to 1000 times lower than that of **4**. This indicates that those wasabi-specific components are much less volatile than **4**. It was believed that the ability of ω-methylthioalkyl ITCs and ω-alkenyl ITCs to activate TRPA1 was equal to that of **4**. However, because of their low volatility, they cannot reach the nasal cavity from the oral cavity to activate TRPA1 expressed in the primary nerve and do not elicit an acrid sensation.

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Naturally occurring TRPA1 and TRPV1 agonists have been mainly identified from spices, and most of them possess strong pungency. Allyl ITC (**4**), cinnamaldehyde (found in cinnamon), CAP (found in chili pepper), and piperine (derived from pepper) are representative agonists for TRPA1 and TRPV1.[7,11,13,20,21] It has been shown that ingestion of TRPA1 and TRPV1 agonists (e.g., compound **4**, piperine, cinnamaldehyde, and CAP) induces adrenaline secretion from adrenal medulla and suppresses body fat accumulation by enhancing energy expenditure.[22−25] Interestingly, some low-stimulant TRPA1 and TRPV1 agonists have been identified, including capsiate (isolated from CH-19 Sweet), miogatrial (a low-pungency component of Japanese ginger), 10-shogaol (found in steamed ginger), and monoacylglycerols (found in wheat).[26−29] Although these agonists have little or no stimulant effects, they activate TRP channels and increase energy consumption similarly to the spicy agonists.[24,30] Hence, the activities to activate TRPA1 of wasabi-specific volatiles (*ω*-methylthioalkyl ITCs and *ω*-alkenyl ITCs) were nearly equal to that of **4**; it is speculated that these ITCs would also promote energy metabolism as **4** does. As weak stimulant TRP agonists would be taken in more easily than irritant agonists would, these low-pungency agonists could be effectively utilized to combat obesity. We hope that this study will also contribute to the development of low-stimulant TRPA1 agonists with antiobesity effects.

EXPERIMENTAL SECTION

Test Compounds. Sixteen ITCs were used for the structure−activity relationship study: isopropyl ITC (**1**), *sec*-butyl ITC (**2**), isobutyl ITC (**3**), allyl ITC (**4**), 3-butenyl ITC (**5**), 4-pentenyl ITC (**6**), 5-hexenyl ITC (**7**), 6-heptenyl ITC (**8**), benzyl ITC (**9**), phenylethyl ITC (**10**), *p*-hydroxybenzyl ITC (**11**), 3-methylthiopropyl ITC (**12**), 4-methylthiobutyl ITC (**13**), 5-methylthiopentyl ITC (**14**), 6-methylthiohexyl ITC (**15**), and 7-methylthioheptyl ITC (**16**). Compounds **1**−**3**, **9**, and **10** were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan), and compound **11** was obtained from Santa Cruz Biotechnology, Inc. (Dallas, TX, USA). Compounds **5**−**8** and **12**−**16** were synthesized, and their purities were examined by gas chromatography (GC). The purities of all ITC compounds were higher than 96%.

HC-030031 was obtained from ChemBridge (San Diego, CA, USA). Capsaicin, capsazepine, and **4** were purchased from Sigma-Aldrich (St. Louis, MO, USA). (−)-Menthol was obtained from Wako Pure Chemical Industries (Osaka, Japan), and 4-(3-chloro-2-pyridinyl)-*N*-[4-(1,1- dimethylethyl)phenyl]-1-piperazinecarboxamide (BCTC) was purchased from Tocris BioScience (Bristol, UK).

Measurement of Purity of ITC Volatiles by Gas Chromatography. An Agilent 6850 series gas chromatograph equipped with a flame ionization detector (FID) (Agilent Technologies, Santa Clara, CA, USA) and a DB-WAX fused silica capillary column (30 m × 0.25 mm i.d.; film thickness of 0.25 μm; J&W Scientific) was used. The measurements were performed using an injector temperature of 210 °C, a helium carrier gas flow rate of 1 mL/min, and an oven temperature of 80 °C increased at 3 °C/min to 210 °C (44 min). The amount of sample injected was 0.2 μL, and a split ratio of 1:100 was used.

Preparation of 3-Butenyl ITC (5), 4-Pentenyl ITC (6), 5-Hexenyl ITC (7), and 6-Heptenyl ITC (8).[31] *N*,*N*-Dimethylformamide (DMF) (480 g, 6.57 mol) was added to a mixture of 4-bromo-1-butene (160 g, 1.19 mol) and potassium thiocyanate (114 g, 1.17 mol), and the mixture was stirred for 1.5 h at 100 °C. After removal of insoluble matter, the mixture was evaporated and 3-butenyl thiocyanate containing DMF (560 g) was obtained. Sodium iodide (208 g, 1.40 mol) and calcium carbonate (11.2 g, 0.11 mol) were added to 3-butenyl thiocyanate, and the mixture was refluxed for 2 h with stirring. Insoluble matter was removed, and the remaining mixture was distilled to afford pure 3-butenyl ITC (**5**) (25.2 g, 0.22 mol, 19%).

Other alkenyl ITCs, 4-pentenyl ITC (**6**), 5-hexenyl ITC (**7**), and 6-heptenyl ITC (**8**), were synthesized from bromo-1-alkenes through the same process utilized for **5**. 5-Bromo-1-pentene (140 g, 0.94 mol) was used to prepare **6** (25.0 g, 0.20 mol, 21%), 6-bromo-1-hexene (120 g, 0.74 mol) was used to synthesize **7** (18.8 g, 0.13 mol, 18%), and 7-bromo-1-heptene (100 g, 0.56 mol) was used to obtain **8** (16.6 g, 0.11 mol, 19%).

Synthesis of 3-Methylthiopropyl ITC (12).[32] Tetra-*n*-butylammonium bromide (50% in water) (39.4 g) was added to a mixture of 1-bromo-3-chloropropane (100 g, 0.635 mol) and methyl mercaptan sodium salt (15% in water) (298 g). The mixture was stirred for 1 h and then extracted with ethyl acetate. The extract was dried with anhydrous sodium sulfate and concentrated under reduced pressure, yielding crude 1-chloro-3-methylthiopropane (88.2 g). Next, DMF (176 g, 2.408 mol) and potassium thiocyanate (85.3 g, 0.877 mol) were heated for 1.5 h at 100 °C with stirring. The reaction mixture was extracted with ethyl acetate, dried with anhydrous sodium sulfate, and concentrated under reduced pressure to afford crude 3-methylthiopropyl thiocyanate (65.8 g). Then, DMF (197 g, 2.70 mol) and tetra-*n*-butylammonium bromide (50% in water) (17.3 g) were added to the residue and refluxed for 1 h. The reaction mixture was extracted with ethyl acetate, dried with anhydrous sodium sulfate, and concentrated under reduced pressure to give 3-methylthiopropyl ITC (**12**) (32.9 g, 0.22 mol, 35%).

Synthesis of 4-Methylthiobutyl ITC (13), 5-Methylthiopentyl ITC (14), 6-Methylthiohexyl ITC (15), and 7-Methylthioheptyl ITC (16).[33] The reaction mixture of **5** (17 g, 0.15 mol) was stirred for 9 h at room temperature with methyl mercaptan (30% in methanol) (72.0 g) and *tert*-butyl hydroperoxide (TBHP) (0.3 g, 0.0033 mol) and then extracted with ethyl acetate. The extract was dried with anhydrous sodium sulfate and evaporated. The residue was distilled and pure 4-methylthiobutyl ITC (**13**) (16.4 g, 0.10 mol, 68%) was obtained.

The other methylthioalkyl ITCs were synthesized from alkenyl ITCs through the same method utilized for **13**. 4-Pentenyl ITC (**6**) (19 g, 0.15 mol) was used to prepare 5-methylthiopentyl ITC (**14**) (16.7 g, 0.10 mol, 64%), 5-hexenyl ITC (**7**) (14.1 g, 0.10 mol) was used to synthesize 6-methylthiohexyl ITC (**15**) (13.6 g, 0.071 mol, 72%), and 6-heptenyl ITC (**8**) (13.0 g, 0.083 mol) was used to obtain 7-methylthioheptyl ITC (**16**) (13.3 g, 0.065 mol, 78%).

Measurement of Activities to Activate TRPA1 and TRPV1 of ITCs Using TRP Channel-Expressing HEK Cells. TRP activity of compounds **1**−**16** was evaluated by a cell-based Ca^{2+} imaging assay using a FlexStation II system (Molecular Devices, Sunnyvale, CA, USA). A detailed experimental method was previously described.[34] In brief, TREx human embryonic kidney (HEK) cells stably expressing human TRPV1 (hTRPV1), hTRPA1, or mouse TRPM8 were used. The HEK cells were seeded in 96-well plates 24 h before each assay, and 1 μg/mL tetracycline was added to the cells to induce the expression of these TRP channels. The HEK cells were loaded with 3 μM Fluo-4 AM (Dojindo Laboratories, Kumamoto, Japan) for 1 h at 37 °C in a measuring buffer, and the change of intracellular Ca^{2+} concentrations was measured by a FlexStation II at 37 °C. Cells were treated with ionomycin (5 μM; positive control) to check viability and to obtain the maximum fluorescence intensity of the cells. All chemicals were administered at a concentration at which they did not show a nonselective effect on the parent cell lines. The following concentrations of agonists (positive controls) and antagonists were used for each TRP channel: 100 μM ally ITC (**4**) and 30 μM HC-030031 for TRPA1; 10 μM CAP and 30 μM CPZ for TRPV1; and 100 μM menthol and 10 μM BCTC for TRPM8. Compounds **1**−**16** and other chemicals were dissolved in DMSO and added to the measuring buffer. Because the ITC compounds are easy to break under aqueous conditions, **1**−**16** were diluted with the measuring buffer just before administration to the cells. Curve fitting and parameter estimations were performed using Prism 5a software (Graph Pad Software, San Diego, CA, USA).

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Calculation of Predicted Vapor Pressure of ITC Compounds. The vapor pressures of the ITCs were quoted from SciFinder (Academic) [https://scifinder.cas.org/scifinder]. The values were calculated using ACD/Laboratories software V11.02 (ACD/Laboratories, Toronto, Ontario, Canada) and displayed in "SciFinder predicted properties, chemical vapor pressure at 25 °C".

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jnatprod.5b00272.

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Notes

The authors declare no competing financial interest.

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