Supplementary Information

Toll-like receptors as a target of food-derived anti-inflammatory compounds

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Supplementary text

The spectral data of isothiocyanate derivatives

3-(Methysulfanyl)propyl isothiocyanate
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.66 (t, J = 6.8 Hz, 2H), 2.62 (t, J = 6.8 Hz, 2H), 2.11 (s, 3H), 1.98 (tt, $J_{b,a}$ = 6.8 Hz, 6.8 Hz, 2H);^{13}C NMR (100 MHz, $CDCl_3$) δ 130.7, 43.7, 30.9, 29.1, 15.6; IR (KBr) 2917, 2853, 2184, 2103, 1442, 1346 (cm^{-1}).
3-(Methysulfinyl)propyl isothiocyanate
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.82-3.68 (m, 2H), 2.88-2.75 (m, 2H), 2.64 (s, 3H), 2.27-2.18 (m, 2H, b); IR (KBr) 2918, 2850, 2185, 2103, 1445, 1350, 1046, 1018 (cm^{-1}).
3-(Methysulfonyl)propyl isothiocyanate
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.79 (t, $J_{a,b}$ = 6.3 Hz, 2H), 3.17 (t, $J_{c,b}$ = 7.2 Hz, 2H), 2.98 (s, 3H), 2.27 (tt, $J_{b,a}$ = 6.3, 7.2 Hz, 2H); IR (KBr) 3017, 2924, 2851, 2189, 2107, 1445, 1412, 1353, 1304, 1131, 963 (cm^{-1}).
3-(1-Propylsulfinyl)propyl isothiocyanate
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.81-3.69 (m, 2H), 2.83-2.60 (m, 4H), 2.26-2.19 (m, 2H), 1.89-1.80 (m, 2H), 1.11 (t, J = 7.3 Hz, 3H); IR (KBr) 2965, 2933, 2875, 2185, 2096, 1456, 1349, 1017 (cm^{-1}).
3-(2-Propylsulfinyl)propyl isothiocyanate
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.84-3.66 (m, 2H, a), 2.89-2.64 (m, 3H, c, d), 2.28-2.18 (m, 2H, b), 1.40-1.21 (m, 6H, e); IR (KBr) 2967, 2932, 2183, 2094, 1447, 1349, 1049, 1014 (cm^{-1}).
3-(Pentylsulfinyl)propyl isothiocyanate
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.83-3.66 (m, 2H), 2.85-2.61 (m, 4H), 2.23 (tt, J = 6.8, 6.8 Hz, 2H), 1.88-1.68 (m, 2H), 1.55-1.32 (m, 4H), 0.95 (t, J = 6.8 Hz, 3H); IR (KBr) 2957, 2931, 2861, 2185, 2098, 1456, 1349, 1042, 1018 (cm^{-1}).
4-(Methylsulfinyl)butyl isothiocyanate
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.61 (t, J = 6.3 Hz, 2H), 2.81-2.65 (m, 2H), 2.61 (s, 3H), 2.02-1.81 (m, 4H); IR (KBr) 2920, 2851, 2106, 2182, 1453, 1350, 1061, 1028 (cm^{-1}).
5-(Methylsulfinyl)pentyl isothiocyanate
^{1}H NMR (400 MHz, $CDCl_3$); 3.56 (t, J = 6.3 Hz, 2H), 2.80-2.62 (m, 2H), 2.59 (s, 3H), 1.90-1.52 (m, 6H); IR (KBr) 3418, 2941, 2863, 2183, 2107, 1455, 1347, 1021 (cm^{-1}).
6-(Methylsulfinyl)hexyl isothiocyanate
^{1}H NMR (400 MHz, $CDCl_3$) δ 3.53 (t, J = 6.3 Hz, 2H), 2.80-2.64 (m, 2H), 2.59 (s, 3H), 1.88-1.44 (m, 8H); IR (KBr) 3433, 2927, 2857, 2182, 2104, 1456, 1348, 1029 (cm^{-1}).
7-(Methylsulfinyl)heptyl isothiocyanate
IR (KBr) 3443, 2931, 2858, 2181, 2105, 1456, 1347, 1036 (cm^{-1}).

8-(Methylsulfinyl)octyl isothiocyanate

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.52 (t, *J* = 6.8 Hz, 2H), 2.79-2.62 (m, 2H), 2.57 (s, 3H), 1.82-1.31 (m, 12H); IR (KBr) 3431, 2929, 2856, 2179, 2106, 1456, 1347, 1039 (cm^{-1}).

4-(1-Methylsulfanyl)butyl isothiocyanate

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.56 (t, *J* = 6.8 Hz, 2H), 2.54 (t, *J* = 6.8 Hz, 2H), 2.11 (s, 3H), 1.86-1.68 (m, 4H); IR (KBr) 2945, 2917, 2857, 2184, 2107, 1450, 1347 (cm^{-1}).

4-(1-Methylsulfonyl)butyl isothiocyanate

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.62 (t, *J* = 6.3 Hz, 2H), 3.07 (t, *J* = 7.3 Hz, 2H), 2.94 (s, 3H), 2.08-1.88 (m, 4H); IR (KBr) 3013, 2929, 2875, 2185, 2111, 1453, 1294, 1130, 966 (cm^{-1}).

Supplementary Table S1

Vegetable name	Botanical nomenclature
Cabbage	*Brassica oleracea L. var. capitata*
Red cabbage	*Brassica oleracea L. var. capitata L. f. rubra (L.) Thell*
Japanese radish	*Raphanus sativus L*
Chinese cabbage	*Brassica rapa var. pekinensis*
Broccoli	*Brassica oleracea var. italica*
Watercress	*Nasturtium officinale*
Kale	*Brassica oleracea var. acephala*
Onion	*Allium cepa L.*
Carrot	*Daucus carota L.*
Yellow carrot	*Daucus carota L.*
Purple carrot	*Daucus carota L.*
Lettuce	*Lactuca sativa L.*
Pumpkin	*Cucurbita maxima*
Tomato	*Solanum lycopersicum*
Egg plant	*Solanum melongena*
Green bell pepper	*Capsicum annuum var. grossum*
Red bell pepper	*Capsicum annuum var. grossum*
Yellow bell pepper	*Capsicum annuum var. grossum*
Celery	*Apium graveolens var. dulce*
Sweet potato	*Ipomoea batatas L.*
Beet	*Beta vulgaris*
Spinach	*Spinacia oleracea L.*

Table. S1. The list of vegetable extracts used in this study.

Supplementary Data Fig. S1

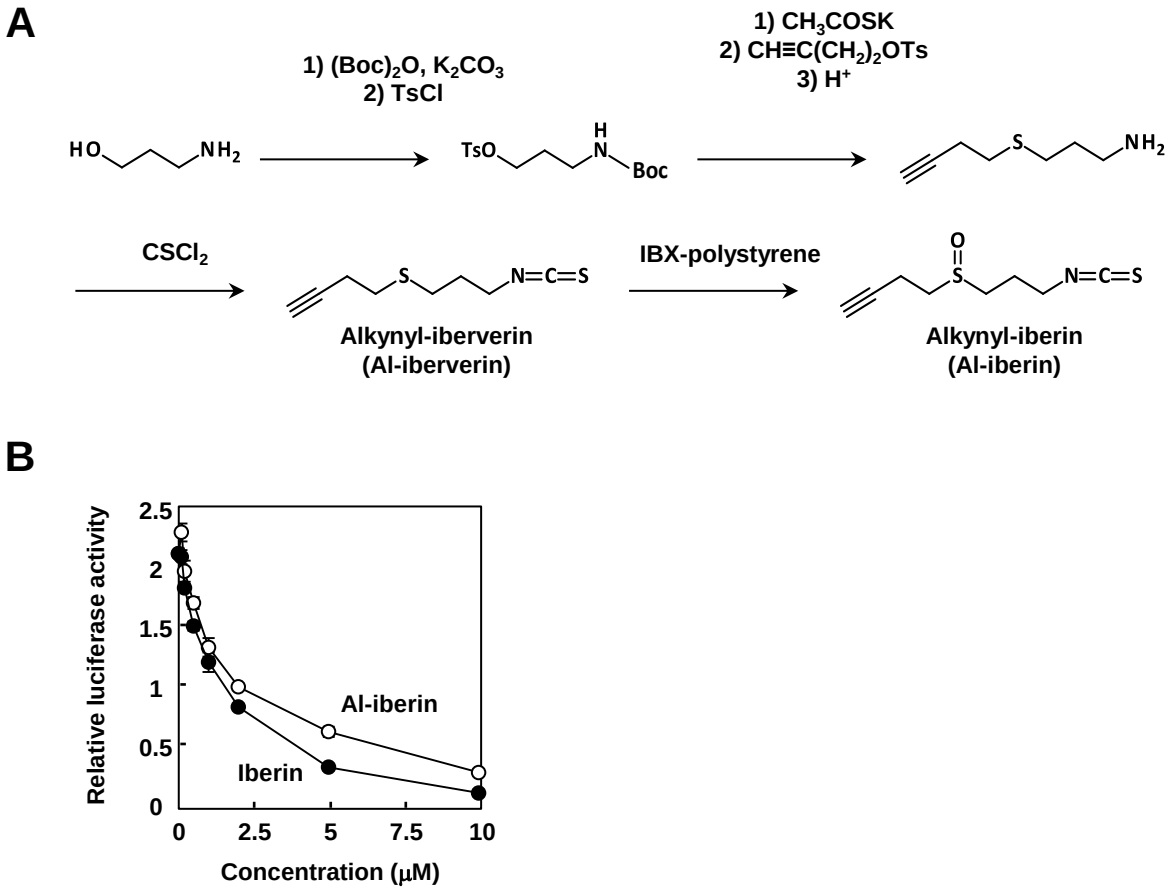


Fig. S1. Click chemistry for detection of iberin-bound proteins.
(A) Synthesis of alkynylated iberin analogues (Al-iberin and Al-iberverin).
(B) Effect of iberin and Al-iberin on LPS-induced TLR4 activity in the HEK293-TLR4 cells.

Supplementary Data Fig. S2

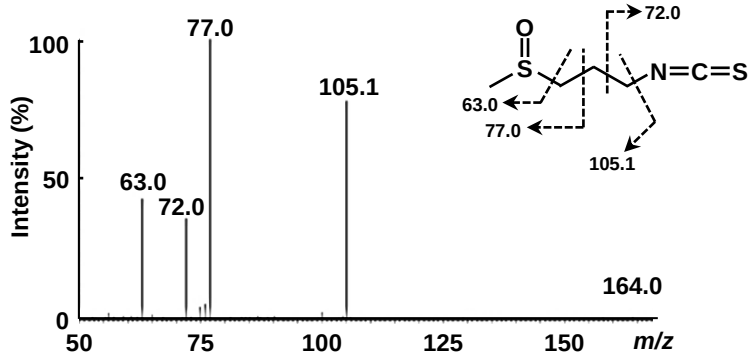
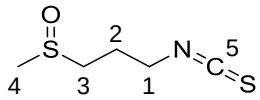


Fig. S2. The MS/MS analysis of iberin isolated from cabbage extract.

Supplementary Data Fig. S3



Position	δ_H (ppm)	δ_C (ppm)
1	*3.752-3.736 (2H, m)*	44.43
2	*2.211 (2H, m)*	23.91
3	*2.805-2.785 (2H, m)*	51.33
4	*2.623 (3H, s)*	38.63-39.29
5		132.48

Fig. S3. The ^{1}H- and ^{13}C NMR analysis of purified iberin.

Supplementary Data Fig. S4

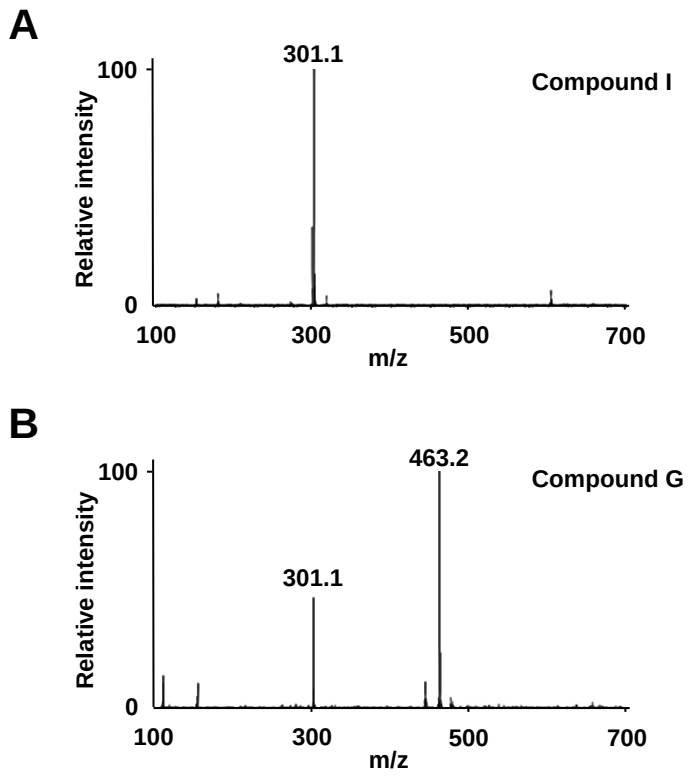
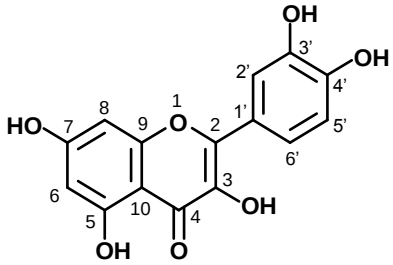


Fig. S4. The mass spectra of compounds G and I.
Compounds I (A) and G (B) were analyzed by UPLC-ESI-MS in the negative ion mode.

Supplementary Data Fig. S5

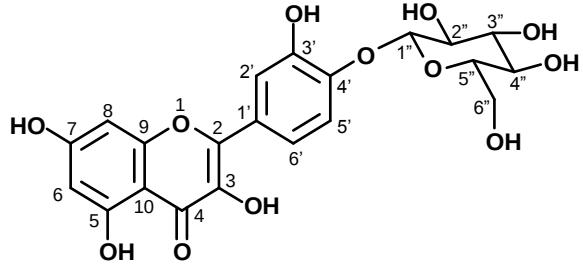
A



Quercetin
(QUE)

Position	δ_H (ppm)	δ_C (ppm)
1		
2		148.00
3		137.21
4		177.33
5		162.50
6	6.27	99.25
7		165.34
8	6.47	94.40
9		158.22
10		104.52
1'		124.15
2'	7.82	115.99
3'		148.75
4'		146.21
5'	6.97	116.22
6'	7.72	121.67

B



Quercetin-4'-O-β-glucoside
(Q4'G)

Position	δ_H (ppm)	δ_C (ppm)
1		
2		148.04
3		137.89
4		177.36
5		162.47
6	6.25	99.31
7		165.66
8	6.44	94.47
9		158.19
10		104.54
1'		127.6
2'	7.81	116.49
3'		147.83
4'		146.79
5'	7.35	117.62
6'	7.75	121.26
1"	5	103.42
2"	3.61	74.82
3"	3.6	77.54
4"	3.53	71.32
5"	3.57	78.36
6"	4.02 3.85	62.44

Fig. S5. The ^{1}H- and ^{13}C NMR analysis of compound I (A) and G (B)

Supplementary Data Fig. S6

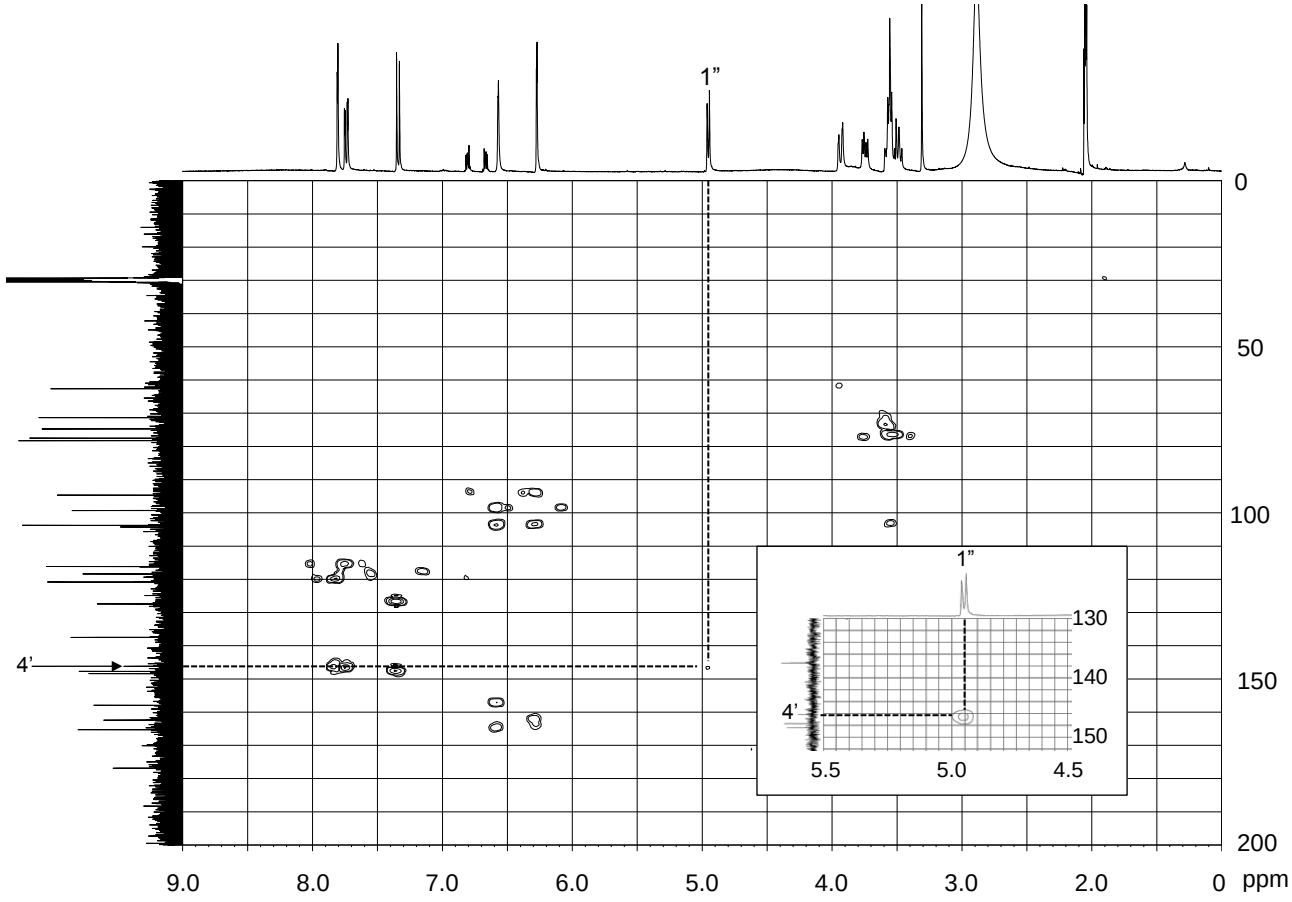


Fig. S6. The HMBC spectrum of compound G.