Synthesis of Benzimidazole Derivatives as Antiallergic Agents with 5-Lipoxygenase Inhibiting Action

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Syntheses were conducted of novel benzimidazole derivatives that suppress histamine release from mast cells, inhibit 5-lipoxygenase, and possess antioxidative action. Among the compounds synthesized, 1-[2-[2-(4-hydroxy-2,3,5-trimethylphenoxy)ethoxy]ethyl]-2-(4-methyl-1-homopiperazino)benzimidazole (22) potently suppressed histamine release from rat peritoneal mast cells triggered by the antigen-antibody reaction, inhibited 5-lipoxygenase in rat basophilic leukemia-1 (RBL-1) cells, and prevented the NADPH-dependent lipid peroxidation induced by Fe^{3+}–ADP in rat liver microsomes, in addition to an antagonizing the contraction of guinea pig ileum caused by histamine.

Key words antiallergic agent; benzimidazole; histamine; 5-lipoxygenase; antioxidative action

In recent years, the rapid increase in the number of patients suffering from a variety of allergic symptoms has become of grave concern. Degranulation of mast cells caused by antigen–antibody reactions triggers type I allergic diseases such as bronchial asthma, allergic rhinitis, atopic dermatitis, and pollenosis. Histamine is one of the chemical mediators released in these allergic reactions and combines with tissue H_1 receptors to produce smooth muscle contraction, vasodilatation, and increased vascular permeability, thereby leading to allergic symptoms.[1)] The mechanism of action of most conventional antiallergic agents is to suppress the release of chemical mediators, with histamine playing a pivotal role, and by antagonizing them. Nevertheless, this action alone is not enough to prevent the symptoms from becoming worse.

Leukotrienes are chemical mediators intimately involved in allergic inflammation and they are able to accumulate in inflammatory cells and contract bronchial smooth muscle.[2,3)] Some drugs developed as peptide leukotriene receptor antagonists have been successfully used clinically.[4,5)] Since 5-lipoxygenase is known to be involved in the biosynthesis of leukotrienes, certain drugs that inhibit this synthesis have also been developed.[6,7)] Moreover, active oxygen is released from inflammatory cells such as eosinophils in allergic inflammation and this has been regarded as one cause of exacerbating symptoms.[8—10)]

We previously synthesized trimethylhydroquinone derivatives with an antihistaminic action, an inhibitory action on 5-lipoxygenase, and an antioxidative action and reported that these compounds are also effective in animal models of asthma.[11,12)] Emedastine, a derivative of benzimidazole, is known as an antiallergic agent with histamine release-suppressing activity and H_1 receptor antagonizing activity.[13—15)] In view of these findings, we have attempted to synthesize antiallergic agents with multiple pharmacological activities by hybridizing benzimidazole derivatives and trimethylhydroquinone derivatives having 5-lipoxygenase inhibiting and antioxidative actions.

In the present paper, we report the synthesis of novel benzimidazole derivatives, their suppression of histamine release from rat peritoneal mast cells, triggered by the antigen–antibody reaction, inhibition of 5-lipoxygenase in rat basophilic leukemia-1 (RBL-1) cells, and prevention of NADPH-dependent lipid peroxidation induced by Fe^{3+}–ADP in rat liver microsomes. We also describe the antagonistic action of representative synthetic compounds on the contraction of guinea pig ileum induced by histamine.

Chemistry

Chart 1 shows the synthetic route for benzimidazole derivatives (**16**—**26**). Compounds **2**—**9** with R1 introduced into 2-chloro-1*H*-benzimidazole (**1**) were synthesized first and they were then allowed to react through condensation with homopiperazine derivatives with R2 introduced to give **16**—**26** (route a, b).

Thus, **1** and halogenated trimethylhydroquinone derivatives[11)] or 2-bromoethylethylether were permitted to react at 80 °C for 3—6 h in *N*,*N*-dimethylformamide (DMF) in the presence of NaH to yield **2**—**9** (route a). Homopiperazine derivatives (**12**—**14**) with trimethylhydroquinone derivatives as R2 were obtained by the reaction of homopiperazine (**10**) with halogenated trimethylhydroquinone derivatives at 100 °C for 1—2 h (route c). **16**—**26** were then obtained by allowing **2**—**9** to react with homopiperazine derivatives (**11**—**14**) at 120—130 °C for 3—15 h (route b). All the reactions gave products in a high yield. **16**—**26** synthesized in this way were converted to their difumarates and their pharmacological activities were examined.

The synthesis of 1-[4-(4-hydroxy-2,3,5-trimethylphenoxy)butyl]-2-(4-methyl-1-homopiperazino)-1*H*-benzimidazole (**18**) was also performed by another route in which the condensation reaction of **1** with homopiperazine derivatives was carried out first and then R1 was introduced into the reaction products (route d, e). Thus, the reaction of **1** with **11** at 130 °C for 6 h yielded 2-(4-methyl-1-homopiperazino)-1*H*-benzimidazole (**15**) (route d) and that of **15** with 4-(4-bromobutoxy)-2,3,6-trimethylphenol in DMF at 100 °C for 18 h in the presence of NaH and NaI gave **18** (route e). However,

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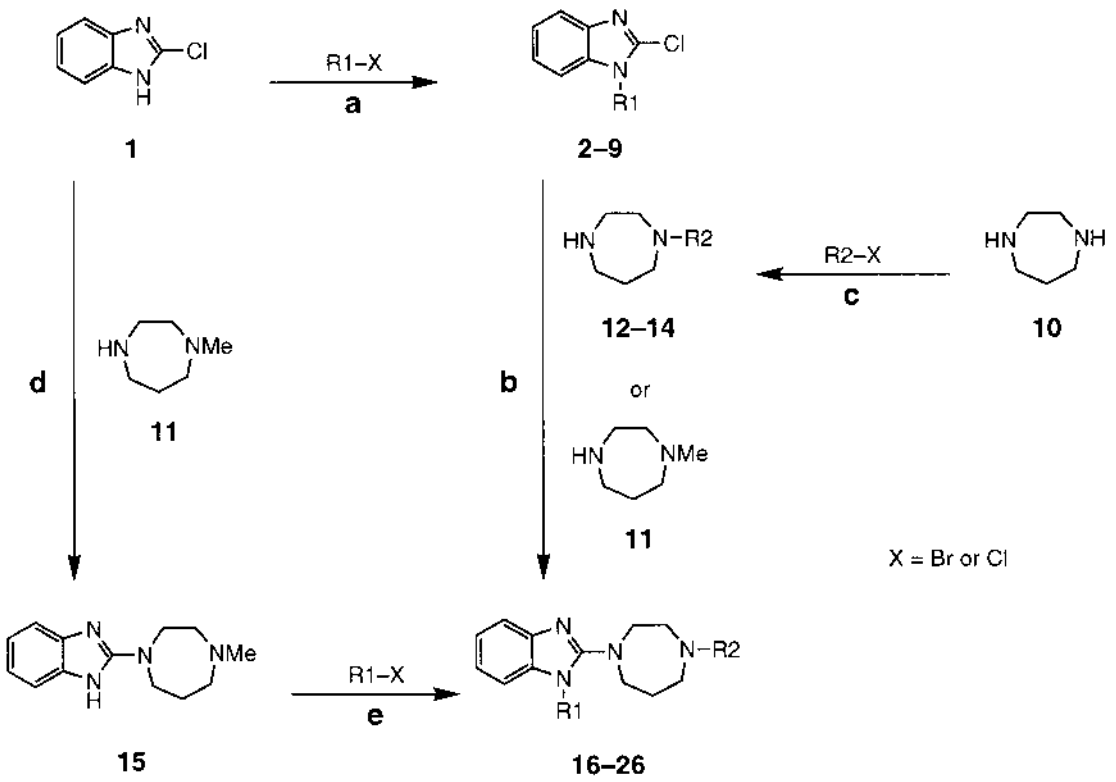


1 2–9 10 11 12–14 11 15 16–26

	R1
2	$-(CH_2)_2O$–Ar–OH
3	$-(CH_2)_3O$–Ar–OH
4	$-(CH_2)_4O$–Ar–OH
5	$-(CH_2)_5O$–Ar–OH

	R1
6	$-(CH_2)_6O$–Ar–OH
7	$-(CH_2)_7O$–Ar–OH
8	$-(CH_2)_2O(CH_2)_2O$–Ar–OH
9	$-(CH_2)_2OCH_2CH_3$

	R2
12	$-(CH_2)_2O$–Ar–OH
13	$-(CH_2)_4O$–Ar–OH
14	$-(CH_2)_2O(CH_2)_2O$–Ar–OH

	R1	R2
16	$-(CH_2)_2O$–Ar–OH	Me
17	$-(CH_2)_3O$–Ar–OH	Me
18	$-(CH_2)_4O$–Ar–OH	Me
19	$-(CH_2)_5O$–Ar–OH	Me
20	$-(CH_2)_6O$–Ar–OH	Me
21	$-(CH_2)_7O$–Ar–OH	Me

	R1	R2
22	$-(CH_2)_2O(CH_2)_2O$–Ar–OH	Me
23	$-(CH_2)_2OCH_2CH_3$	$-(CH_2)_4O$–Ar–OH
24	$-(CH_2)_2OCH_2CH_3$	$-(CH_2)_2O(CH_2)_2O$–Ar–OH
25	$-(CH_2)_2O$–Ar–OH	$-(CH_2)_2O$–Ar–OH
26	$-(CH_2)_4O$–Ar–OH	$-(CH_2)_4O$–Ar–OH

Chart 1

Table 1. Effect of Benzimidazole Derivatives on Histamine Release from Sensitized Rat Peritoneal Mast Cells Produced by Antigen

Compd.	Inhibition (%)[a)]
16	34.2
17	27.8
18	25.7
19	58.4
20	15.5
21	45.0
22	66.8
23	21.2
24	33.2
25	51.5
26	44.9
Emedastine	48.8

a) Test compound concentration, 10^{-5} M. All test compounds were used as difumarate salts. Results are the means of duplicate determinations.

the reactions along this synthetic route gave **15** and **18** in rather low yield, 38% and 23%, respectively.

Pharmacological Results and Discussion

The inhibitory action of benzimidazole derivatives (**16**—**26**) was examined on histamine release from rat peritoneal mast cells induced by antigen–antibody reaction. Mast cells were withdrawn from the peritoneal cavity of rats passively sensitized with anti-2,4-dinitrophenyl-bovine serum albumin (DNP-BSA) serum. After incubating the cells with test compound for 15 min, degranulation was induced by incubating the cells for 20 min in the presence of added DNP-BSA as the antigen. The amount of histamine within the cells together with that released from the cells was determined fluorophotometrically using *o*-phthalaldehyde as the reagent, the rate of inhibition of histamine release by the test compound *versus* the control was calculated from the analytical data obtained. All the synthetic benzimidazole derivatives and emedastine exhibited histamine release inhibiting activity at a concentration of 10^{-5} M (Table 1). **22** had the highest activity among the benzimidazole derivatives synthesized while **19** and **25** were more effective than emedastine in suppressing histamine release. This suggests that introduction of a trimethylhydroquinone moiety has little or no effect on the inhibition of histamine release by benzimidazole derivatives.

The inhibitory action of these synthetic compounds was then examined on 5-lipoxygenase from RBL-1 cells. All synthetic benzimidazole derivatives, and nordihydroguaiaretic acid (NDGA) used as the positive control, inhibited 5-lipoxygenase at a concentration of 10^{-6} M (Table 2). The action of **25** and **26** was potent, both having two trimethylhydroquinone moieties in their structure, among the benzimidazole derivatives, and their action was as strong as that of NDGA with two catechol moieties. Emedastine produced no inhibition at this concentration. Hence, the introduction of a trimethylhydroquinone moiety into the benzimidazole derivatives confers 5-lipoxygenase inhibiting activity.

The Fe^{3+}–ADP-induced NADPH-dependent lipid peroxidation inhibition produced by the synthetic compounds in rat liver microsomes was examined as an index of their antioxidative action. Most of the compounds showed inhibition at a concentration of 3×10^{-6} M and their activity was higher than that of butylated hydroxytoluene (BHT) used as a positive control (Table 3). No activity was exhibited by **17**, **21** and

Table 2. Effect of Benzimidazole Derivatives and NDGA on RBL-1 Cell 5-Lipoxygenase Activity

Compd.	Inhibition (%)[a)]
16	41.3
17	28.4
18	65.9
19	76.0
20	68.7
21	16.5
22	73.7
23	66.9
24	65.1
25	83.7
26	87.6
Emedastine	3.2
NDGA	84.8

a) Test compound concentration, 10^{-6} M. **16**—**26** and emedastine were used as difumarate salts. Results are the means of duplicate determinations.

Table 3. Effect of Benzimidazole Derivatives and BHT on Fe^{3+}–ADP-Induced NADPH-Dependent Lipid Peroxidation in Rat Liver Microsomes

Compd.	Inhibition (%)[a)]
16	18.9
17	−3.8
18	90.2
19	88.8
20	87.4
21	−5.0
22	42.8
23	86.9
24	88.5
25	87.4
26	92.1
Emedastine	−1.5
BHT	17.0

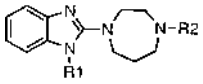
a) Test compound concentration, 3×10^{-6} M. **16**—**26** and emedastine were used as difumarate salts. Results are the means of duplicate determinations.

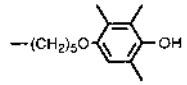
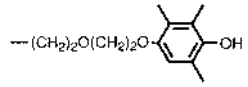
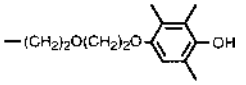
emedastine at this concentration. So, most of the benzimidazole derivatives have antioxidizing activity as well as a 5-lipoxygenase inhibiting activity.

Emedastine exhibits potent H_1 receptor antagonism, in addition to inhibiting histamine release from mast cells. Therefore, the H_1 receptor antagonism of the synthetic benzimidazole derivatives was examined using the contraction of guinea pig ileum induced by histamine. Guinea pig ileum was incubated for 5 min with test compound and the pA_2 value was determined after cumulative histamine administration to the incubated ileum. The results are shown in Table 4. When the activity of **19** was compared with that of **22**, both of which have a trimethylhydroquinone moiety introduced in R1, the latter with two ether groups as spacers was found to exhibit antagonism, whereas the former was inactive at 10^{-6} M. Similarly, **24** was also inactive although it has the same moiety introduced in R2 as that in R1 of **22**. These findings suggest that the introduction of a trimethylhydroquinone moiety into R2 does not retain the H_1 receptor antagonism of the benzimidazole derivatives while its introduction into R1 is not necessarily effective.

In summary, we have synthesized novel benzimidazole derivatives and demonstrated that they have multiple antiallergic pharmacological activities. These compounds may play a

Table 4. Effect of Benzimidazole Derivatives on Histamine-Induced Contraction of Isolated Guinea Pig Ileum



Compd.	R1	R2	pA_2[a]
19		Me	<6.00
22		Me	7.49
24	$-(CH_2)_2OCH_2CH_3$		<6.00
Emedastine	$-(CH_2)_2OCH_2CH_3$	Me	9.09

a) Test compound concentration, 10^{-6}—10^{-9} M. All test compounds were used as difumarate salts. Results are the means of triplicate determinations.

major role in the futuredevelopment of new antiallergic agents.

Experimental

Melting points were measured on an Ishii Shoten Co., Ltd. MP-1 apparatus and are uncorrected. ^{1}H-NMR spectra were measured on a JEOL JNM-EX270 (270 MHz) spectrometer in $CDCl_3$ solution with tetramethylsilane as internal standard. FAB-MS were obtained on a JEOL JMS-AX505H spectrometer.

2-Chloro-1-[2-(4-hydroxy-2,3,5-trimethylphenoxy)ethyl]-1*H*-benzimidazole (2) 2-Chloro-1*H*-benzimidazole (**1**) (1.8 g, 11.8 mmol) was dissolved in 10 ml DMF. NaH (0.520 g, 11.8 mmol) was added to the solution in an ice-bath and stirred for 30 min. To the mixture was added 4-(2-bromoethoxy)-2,3,6-trimethylphenol (3.1 g, 11.9 mmol) followed by stirring at 80 °C for 4 h. The mixture was then added to water, extracted with AcOEt, washed with water and brine, dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue was washed with hexane–AcOEt (1 : 1) to afford **2** (2.7 g, 8.2 mmol, 69%) as colorless crystals, mp 192—195 °C. ^{1}H-NMR δ: 1.92 (3H, s), 2.10 (3H, s), 2.17 (3H, s), 4.22 (2H, t, J=5.61 Hz), 4.49 (1H, s), 4.59 (2H, d, J=5.28 Hz), 6.40 (1H, s), 7.24—7.32 (2H, m), 7.37—7.41 (1H, m), 7.69—7.71 (1H, m). FAB-MS *m/z*: 331 (M^++1).

2-Chloro-1-[3-(4-hydroxy-2,3,5-trimethylphenoxy)propyl]-1*H*-benzimidazole (3) **1** (1.6 g, 10.5 mmol) was dissolved in 10 ml DMF. NaH (0.584 g, 10.5 mmol) was added to the solution in an ice-bath and stirred for 30 min. To the mixture was added 4-(3-bromopropyloxy)-2,3,6-trimethylphenol (2.8 g, 10.5 mmol) followed by stirring at 80 °C for 4 h. The mixture was then added to water, extracted with AcOEt, washed with water and brine, dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue was washed with hexane–AcOEt (1 : 1) to afford **3** (2.4 g, 7.0 mmol, 67%) as colorless crystals, mp 114—115 °C. ^{1}H-NMR δ: 2.20—2.30 (2H, m), 2.12 (3H, s), 2.17 (3H, s), 2.21 (3H, s), 3.92 (2H, t, J=6.27 Hz), 4.26 (2H, t, J=7.26 Hz), 4.36 (1H, s), 6.48 (1H, s), 7.22—7.32 (3H, m), 7.64—7.70 (1H, m). FAB-MS *m/z*: 345 (M^++1).

2-Chloro-1-[4-(4-hydroxy-2,3,5-trimethylphenoxy)butyl]-1*H*-benzimidazole (4) **1** (2.0 g, 13 mmol) was dissolved in 30 ml DMF. NaH (0.576 g, 14 mmol) was added to the solution in an ice-bath and stirred for 30 min. To the mixture was added 4-(4-bromobutoxy)-2,3,6-trimethylphenol (3.7 g, 13 mmol) followed by stirring at 80 °C for 4 h. The mixture was then added to water, extracted with AcOEt, washed with water and brine, dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue was washed with hexane–AcOEt (1 : 1) to afford **4** (3.9 g, 10.9 mmol, 83%) as pale yellow crystals, mp 121—123 °C. ^{1}H-NMR δ: 1.80—1.88 (2H, m), 2.01—2.13 (2H, m), 2.11 (3H, s), 2.17 (3H, s), 2.21 (3H, s), 3.90 (2H, t, J=5.94 Hz), 4.28 (2H, t, J=7.26 Hz), 4.51 (1H, s), 6.47 (1H, s), 7.23—7.32 (3H, m), 7.64—7.71 (1H, m). FAB-MS *m/z*: 359 (M^++1).

2-Chloro-1-[5-(4-hydroxy-2,3,5-trimethylphenoxy)pentyl]-1*H*-benzimidazole (5) **1** (5.5 g, 36 mmol) was dissolved in 50 ml DMF. NaH (1.6 g, 39 mmol) was added to the solution in an ice-bath and stirred for 30 min. To the mixture was added 4-(5-bromopentyloxy)-2,3,6-trimethylphenol (11.5 g, 36 mmol) followed by stirring at 80 °C for 4 h. The mixture was then added to water, extracted with AcOEt, washed with water and brine, dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue was washed with hexane–AcOEt (1 : 1) to afford **5** (11.7 g, 31.4 mmol, 87%) as pale yellow crystals, mp 131—133 °C. ^{1}H-NMR δ: 1.56—1.60 (2H, m), 1.76—1.97 (4H, m), 2.09 (3H, s), 2.16 (3H, s), 2.21 (3H, s), 3.85 (2H, t, J=5.94 Hz), 4.22 (2H, t, J=6.93 Hz), 4.35 (1H, s), 6.48 (1H, s), 7.24—7.30 (3H, m), 7.66—7.71 (1H, m). FAB-MS *m/z*: 373 (M^++1).

2-Chloro-1-[6-(4-hydroxy-2,3,5-trimethylphenoxy)hexyl]-1*H*-benzimidazole (6) **1** (1.5 g, 9.8 mmol) was dissolved in 10 ml DMF. NaH (0.432 g, 9.8 mmol) was added to the solution in an ice-bath and stirred for 30 min. To the mixture was added 4-(6-bromohexyloxy)-2,3,6-trimethylphenol (3.1 g, 9.8 mmol) followed by stirring at 80 °C for 4 h. The mixture was then added to water, extracted with AcOEt, washed with water and brine, dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue was washed with hexane–AcOEt (1 : 1) to afford **6** (2.9 g, 7.5 mmol, 77%) as pale yellow crystals, mp 106—108 °C. ^{1}H-NMR δ: 1.40—1.59 (4H, m), 1.71—1.89 (4H, m), 2.12 (3H, s), 2.17 (3H, s), 2.21 (3H, s), 3.85 (2H, t, J=6.27 Hz), 4.19 (2H, t, J=7.26 Hz), 4.41 (1H, s), 6.49 (1H, s), 7.24—7.31 (3H, m), 7.66—7.71 (1H, m). FAB-MS *m/z*: 387 (M^++1).

2-Chloro-1-[7-(4-hydroxy-2,3,5-trimethylphenoxy)heptyl]-1*H*-benzimidazole (7) **1** (1.5 g, 9.8 mmol) was dissolved in 10 ml DMF. NaH (0.545 g, 9.8 mmol) was added to the solution in an ice-bath and stirred for 30 min. To the mixture was added 4-(7-bromoheptyloxy)-2,3,6-trimethylphenol (3.2 g, 9.8 mmol) followed by stirring at 80 °C for 4 h. The mixture was then added to water, extracted with AcOEt, washed with water and brine, dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue was washed with hexane–AcOEt (1 : 1) to afford **7** (2.9 g, 7.2 mmol, 74%) as colorless crystals, mp 123—125 °C. ^{1}H-NMR δ: 1.38—1.58 (6H, m), 1.74—1.98 (4H, m), 2.08 (3H, s), 2.17 (3H, s), 2.21 (3H, s), 3.86 (2H, t, J=5.92 Hz), 4.27 (2H, t, J=7.26 Hz), 4.36 (1H, s), 6.44 (1H, s), 7.24—7.31 (3H, m), 7.63—7.70 (1H, m). FAB-MS *m/z*: 401 (M^++1).

2-Chloro-1-[2-[2-(4-hydroxy-2,3,5-trimethylphenoxy)ethoxy]ethyl]-1*H*-benzimidazole (8) **1** (20 g, 131 mmol) was dissolved in 80 ml DMF. NaH (7.3 g, 131 mmol) was added to the solution in an ice-bath and stirred for 30 min. To the mixture was added 4-[2-(2-chloroethoxy)ethoxy]-2,3,6-trimethylphenol (33.9 g, 131 mmol) followed by stirring at 80 °C for 4 h. The mixture was then added to water, extracted with AcOEt, washed with water and brine, dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue was washed with hexane–AcOEt (1 : 1) to afford **8** (37.5 g, 100 mmol, 76%) as colorless crystals, mp 104—105 °C. ^{1}H-NMR δ: 2.08 (3H, s), 2.16 (3H, s), 2.20 (3H, s), 3.70—3.75 (2H, m), 3.88—3.94 (4H, m), 4.40 (2H, t, J=5.61 Hz), 6.44 (1H, s), 7.23—7.29 (2H, m), 7.37—7.41 (1H, m), 7.66—7.69 (1H, m). FAB-MS *m/z*: 375 (M^++1).

2-Chloro-1-[2-(ethoxy)ethyl]-1*H*-benzimidazole (9) **1** (6.0 g, 39 mmol) was dissolved in 50 ml DMF. NaH (1.6 g, 39 mmol) was added to the solution in an ice-bath and stirred for 1 h. To the mixture was added 2-bromoethylethether (6.0 g, 39 mmol) followed by stirring at room temperature for 3 h. The mixture was then added to cold water, extracted with ether, washed with water and brine, dried over anhydrous Na_2SO_4, evaporated and chromatographed on silica gel ($CHCl_3$–MeOH, 50 : 1) to afford **9** (4.7 g, 20.9 mmol, 53%) as a colorless oil. ^{1}H-NMR δ: 1.11 (3H, t, J=7.26 Hz), 3.43 (2H, q, J=6.93 Hz), 3.74 (2H, t, J=5.94 Hz), 4.36 (2H, t, J=5.94 Hz), 7.23—7.35 (2H, m), 7.37—7.41 (1H, m), 7.65—7.71 (1H, m). FAB-MS *m/z*: 225 (M^++1).

4-[2-(1-Homopiperazino)ethoxy]-2,3,6-trimethylphenol (12) 4-(2-Bromoethoxy)-2,3,6-trimethylphenol (4.0 g, 15.4 mmol) and homopiperazine (**10**) (4.6 g, 46 mmol) were mixed at 100 °C for 2 h. The mixture was then added to water, extracted with $CHCl_3$, washed with water and brine, dried over anhydrous Na_2SO_4, evaporated and chromatographed on silica gel ($CHCl_3$–MeOH, 20 : 1) to afford **12** (4.2 g, 15.1 mmol, 98%) as a brown oil. ^{1}H-NMR δ: 1.73—1.80 (2H, m), 2.13 (3H, s), 2.16 (3H, s), 2.21 (3H, s), 2.80—2.99 (10H, m), 3.95 (2H, d, J=5.94 Hz), 6.52 (1H, s). FAB-MS *m/z*: 279 (M^++1).

4-[4-(1-Homopiperazino)butoxy]-2,3,6-trimethylphenol (13) 4-(4-Bromobutoxy)-2,3,6-trimethylphenol (5.0 g, 17.5 mmol) and **10** (5.2 g, 52 mmol) were mixed at 100 °C for 2 h. The mixture was then added to water, extracted with $CHCl_3$, washed with water and brine, dried over anhydrous Na_2SO_4, evaporated and chromatographed on silica gel ($CHCl_3$–MeOH, 20 : 1) to afford **13** (4.7 g, 15.3 mmol, 87%) as a brown oil. ^{1}H-NMR δ: 1.61—1.69 (2H, m), 1.71—1.82 (4H, m), 2.14 (3H, s), 2.17 (3H, s), 2.21

(3H, s), 2.64—2.76 (6H, m), 3.58—3.66 (4H, m), 3.90 (2H, d, J=5.94 Hz), 4.32 (1H, s), 6.52 (1H, s). FAB-MS *m/z*: 307 (M^++1).

4-[2-[2-(1-Homopiperazino)ethoxy]ethoxy]-2,3,6-trimethylphenol (14) 4-[2-(2-Chloroethoxy)ethoxy]-2,3,6-trimethylphenol (4.0 g, 15.5 mmol) and **10** (4.6 g, 46 mmol) were mixed at 100 °C for 1.5 h. The mixture was then added to water, extracted with $CHCl_3$, washed with water and brine, dried over anhydrous Na_2SO_4, evaporated and chromatographed on silica gel ($CHCl_3$–MeOH, 20 : 1) to afford **14** (4.7 g, 14.6 mmol, 94%) as a brown oil. ^{1}H-NMR δ: 1.70—1.79 (2H, m), 2.14 (3H, s), 2.16 (3H, s), 2.21 (3H, s), 2.73—2.81 (6H, m), 3.87—3.93 (4H, m), 3.66 (2H, t, J=5.94 Hz), 3.78 (2H, t, J=5.28 Hz), 4.02 (2H, t, J=4.61 Hz), 6.53 (1H, s). FAB-MS *m/z*: 323 (M^++1).

2-(4-Methyl-1-homopiperazino)-1*H*-benzimidazole (15) **1** (12 g, 78 mmol) and 1-methylhomopiperazine (**11**, 22 g, 192 mmol) were mixed at 130 °C for 6 h. The mixture was then added to water, extracted with AcOEt, washed with water and brine, dried over anhydrous Na_2SO_4, and evaporated to dryness. The residue was recrystallized from EtOH–AcOEt to afford **15** (6.8 g, 29.4 mmol, 38%) as pale brown crystals, mp 193—195 °C. ^{1}H-NMR δ: 1.97—2.06 (2H, m), 2.36 (3H, s), 2.58—2.62 (2H, m), 2.69—2.72 (2H, m), 3.70 (2H, t, J=5.94 Hz), 3.80—3.82 (2H, m), 7.03 (2H, br), 7.26 (2H, br). FAB-MS *m/z*: 231 (M^++1).

1-[2-(4-Hydroxy-2,3,5-trimethylphenoxy)ethyl]-2-(4-methyl-1-homopiperazino)-1*H*-benzimidazole (16) **2** (1.0 g, 3.0 mmol) and **11** (1.5 g, 13.2 mmol) were mixed at 130 °C for 14 h. The mixture was chromatographed on silica gel ($CHCl_3$–MeOH, 50 : 1) to afford **16** (1.0 g, 2.4 mmol, 81%) as a brown oil. ^{1}H-NMR δ: 1.96—2.04 (2H, m), 2.67—2.78 (2H, m), 1.98 (3H, s), 2.13 (3H, s), 2.17 (3H, s), 2.38 (3H, s), 3.62—3.69 (2H, m), 3.99 (2H, t, J=5.61 Hz), 4.19—4.24 (4H, m), 4.41 (2H, t, J=5.94 Hz), 6.42 (1H, s), 7.10—7.18 (3H, m), 7.53 (1H, d, J=7.25 Hz). FAB-MS *m/z*: 409 (M^++1).

1-[3-(4-Hydroxy-2,3,5-trimethylphenoxy)propyl]-2-(4-methyl-1-homopiperazino)-1*H*-benzimidazole (17) **3** (1.0 g, 2.9 mmol) and **11** (0.87 g, 7.6 mmol) were mixed at 130 °C for 6 h. The mixture was chromatographed on silica gel ($CHCl_3$–MeOH, 50 : 1) to afford **17** (1.0 g, 2.4 mmol, 82%) as a brown oil. ^{1}H-NMR δ: 1.78—2.06 (2H, m), 2.08—2.16 (2H, m), 2.08 (3H, s), 2.17 (3H, s), 2.22 (3H, s), 2.38 (3H, s), 2.64—2.74 (4H, m), 3.58—3.64 (4H, m), 3.92 (2H, t, J=5.94 Hz), 4.00 (2H, t, J=7.58 Hz), 4.52 (1H, s), 6.44 (1H, s), 7.10—7.18 (3H, m), 7.62—7.72 (1H, m). FAB-MS *m/z*: 423 (M^++1).

1-[4-(4-Hydroxy-2,3,5-trimethylphenoxy)butyl]-2-(4-methyl-1-homopiperazino)-1*H*-benzimidazole (18) by Route b **4** (1.2 g, 3.4 mmol) and **11** (1.9 g, 16.7 mmol) were mixed at 130 °C for 14 h. The mixture was chromatographed on silica gel ($CHCl_3$–MeOH, 50 : 1) to afford **18** (1.2 g, 2.8 mmol, 82%) as a brown oil. ^{1}H-NMR δ: 1.76—1.84 (2H, m), 1.93—2.03 (4H, m), 2.07 (3H, s), 2.16 (3H, s), 2.21 (3H, s), 2.37 (3H, s), 2.66—2.77 (4H, m), 3.60—3.64 (4H, m), 3.90 (2H, t, J=5.94 Hz), 4.08 (2H, t, J=7.59 Hz), 6.47 (1H, s), 7.07—7.19 (3H, m), 7.53 (1H, dd, J=1.32, 6.62 Hz). FAB-MS *m/z*: 437 (M^++1).

18 by Route e **15** (2.3 g, 10 mmol) was dissolved in 10 ml DMF. NaH (0.44 g, 11 mmol) was added to the solution in an ice-bath and stirred for 1 h. To the mixture was added 4-(4-bromobutoxy)-2,3,6-trimethylphenol (3.1 g, 10 mmol), NaI (0.3 g, 2 mmol) followed by stirring at 100 °C for 18 h. The mixture was then added to water, extracted with $CHCl_3$, washed with water and brine, dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue was chromatographed on silica gel ($CHCl_3$–MeOH, 50 : 1) to afford **18** (1.0 g, 2.3 mmol, 23%) as a brown oil.

1-[5-(4-Hydroxy-2,3,5-trimethylphenoxy)pentyl]-2-(4-methyl-1-homopiperazino)-1*H*-benzimidazole (19) **5** (6.6 g, 17.7 mmol) and **11** (10.2 g, 89.3 mmol) were mixed at 130 °C for 5 h. The mixture was chromatographed on silica gel ($CHCl_3$–MeOH, 50 : 1) to afford **19** (7.2 g, 16.0 mmol, 90%) as a brown oil. ^{1}H-NMR δ: 1.48—1.54 (2H, m), 1.76—1.91 (4H, m), 1.96—2.05 (2H, m), 2.11 (3H, s), 2.17 (3H, s), 2.21 (3H, s), 2.40 (3H, s), 2.69—2.78 (4H, m), 3.58—3.64 (4H, m), 3.86 (2H, t, J=6.27 Hz), 4.01 (2H, t, J=7.58 Hz), 6.48 (1H, s), 7.11—7.18 (3H, m), 7.53 (1H, dd, J=1.98, 6.92 Hz). FAB-MS *m/z*: 451 (M^++1).

1-[6-(4-Hydroxy-2,3,5-trimethylphenoxy)hexyl]-2-(4-methyl-1-homopiperazino)-1*H*-benzimidazole (20) **6** (1.0 g, 2.6 mmol) and **11** (0.78 g, 6.8 mmol) were mixed at 130 °C for 5 h. The mixture was chromatographed on silica gel ($CHCl_3$–MeOH, 50 : 1) to afford **20** (1.1 g, 2.4 mmol, 92%) as a brown oil. ^{1}H-NMR δ: 1.39—1.47 (2H, m), 1.50—1.58 (2H, m), 1.71—1.89 (4H, m), 2.00—2.11 (2H, m), 2.13 (3H, s), 2.17 (3H, s), 2.21 (3H, s), 2.41 (3H, s), 2.70—2.80 (4H, m), 3.59—3.65 (4H, m), 3.86 (2H, t, J=6.26 Hz), 3.99 (2H, t, J=7.92 Hz), 6.50 (1H, s), 7.09—7.18 (3H, m), 7.53 (1H, dd, J=1.32, 6.26 Hz). FAB-MS *m/z*: 465 (M^++1).

1-[7-(4-Hydroxy-2,3,5-trimethylphenoxy)heptyl]-2-(4-methyl-1-homopiperazino)-1*H*-benzimidazole (21) **7** (1.0 g, 2.5 mmol) and **11** (0.75 g, 6.6 mmol) were mixed at 130 °C for 6 h. The mixture was chromatographed on silica gel ($CHCl_3$–MeOH, 50 : 1) to afford **21** (0.94 g, 2.0 mmol, 80%) as a brown oil. ^{1}H-NMR δ: 1.44—1.58 (4H, m), 1.72—1.91 (5H, m), 1.94—2.04 (4H, m), 2.10 (3H, s), 2.17 (3H, s), 2.22 (3H, s), 2.40 (3H, s), 2.69—2.77 (4H, m), 3.59—3.64 (4H, m), 3.86 (2H, t, J=6.22 Hz), 3.98 (2H, t, J=7.72 Hz), 6.48 (1H, s), 7.11—7.17 (3H, m), 7.52 (1H, dd, J=1.90, 6.64 Hz). FAB-MS *m/z*: 479 (M^++1).

1-[2-[2-(4-Hydroxy-2,3,5-trimethylphenoxy)ethoxy]ethyl]-2-(4-methyl-1-homopiperazino)-1*H*-benzimidazole (22) **8** (20.0 g, 5.3 mmol) and **11** (16.0 g, 14.0 mmol) were mixed at 130 °C for 8 h. The mixture was chromatographed on silica gel ($CHCl_3$–MeOH, 50 : 1) to afford **22** (22.0 g, 4.9 mmol, 91%) as a pale yellow oil. ^{1}H-NMR δ: 1.92—2.01 (2H, m), 2.09 (3H, s), 2.15 (3H, s), 2.20 (3H, s), 2.38 (3H, s), 2.64—2.76 (4H, m), 3.60—3.67 (4H, m), 3.74—3.77 (2H, m), 3.91—3.99 (4H, m), 4.22 (2H, t, J=6.27 Hz), 6.48 (1H, s), 7.08—7.17 (2H, m), 7.24—7.27 (1H, m), 7.52 (1H, dd, J=1.32, 7.58 Hz). FAB-MS *m/z*: 453 (M^++1).

1-(2-Ethoxyethyl)-2-[4-[4-(4-hydroxy-2,3,5-trimethylphenoxy)butyl]-1-homopiperazino]-1*H*-benzimidazole (23) **9** (0.8 g, 3.5 mmol) and **13** (1.0 g, 3.3 mmol) were mixed at 120 °C for 15 h. The mixture was chromatographed on silica gel ($CHCl_3$–MeOH, 50 : 1) to afford **23** (1.2 g, 2.4 mmol, 73%) as a brown oil. ^{1}H-NMR δ: 1.16 (3H, t, J=7.24 Hz), 1.68—1.87 (1H, m), 1.99—2.09 (2H, m), 2.14 (3H, s), 2.17 (3H, s), 2.22 (3H, s), 2.59 (2H, t, J=5.61 Hz), 2.77—2.87 (4H, m), 3.47 (2H, q, J=6.72 Hz), 3.60—3.68 (4H, m), 3.76—3.83 (2H, m), 3.88—3.95 (4H, m), 4.10—4.23 (2H, m), 4.16 (2H, t, J=6.26 Hz), 6.54 (1H, s), 7.09—7.13 (2H, m), 7.24—7.28 (1H, m), 7.53 (1H, d, J=7.58 Hz). FAB-MS *m/z*: 495 (M^++1).

1-(2-Ethoxyethyl)-2-[4-[2-[2-(4-hydroxy-2,3,5-trimethylphenoxy)-ethoxy]ethyl]-1-homopiperazino]-1*H*-benzimidazole (24) **9** (0.8 g, 3.5 mmol) and 1**4** (1.1 g, 3.4 mmol) were mixed at 120 °C for 15 h. The mixture was chromatographed on silica gel ($CHCl_3$–MeOH, 50 : 1) to afford **24** (1.2 g, 2.4 mmol, 71%) as a brown oil. ^{1}H-NMR δ: 1.14 (3H, t, J=7.26 Hz), 1.93—1.98 (1H, m), 2.14—2.17 (2H, m), 2.14 (3H, s), 2.15 (3H, s), 2.21 (3H, s), 2.76—2.92 (6H, m), 3.45 (2H, q, J=6.93 Hz), 3.57—3.65 (4H, m), 3.68 (2H, t, J=5.94 Hz), 3.74—3.81 (4H, m), 4.02—4.06 (2H, m), 4.16 (2H, t, J=6.26 Hz), 6.54 (1H, s), 7.09—7.14 (2H, m), 7.23—7.26 (1H, m), 7.58 (1H, dd, J=1.65, 4.94 Hz). FAB-MS *m/z*: 511 (M^++1).

1-[2-(4-Hydroxy-2,3,5-trimethylphenoxy)ethyl]-2-[4-[2-(4-hydroxy-2,3,5-trimethylphenoxy)ethyl]-1-homopiperazino]-1*H*-benzimidazole (25) **2** (1.6 g, 4.8 mmol) and **12** (1.4 g, 5.0 mmol) were mixed at 120 °C for 15 h. The mixture was chromatographed on silica gel ($CHCl_3$–MeOH, 50 : 1) to afford **25** (1.8 g, 3.1 mmol, 65%) as a brown oil. ^{1}H-NMR δ: 1.94—2.08 (2H, m), 1.98 (3H, s), 2.12 (6H, s), 2.15 (3H, s), 2.17 (3H, s), 2.21 (3H, s), 2.90—3.00 (6H, m), 3.62—3.68 (4H, m), 4.00 (2H, t, J=5.61 Hz), 4.22 (2H, t, J=5.61 Hz), 4.41 (2H, t, J=5.94 Hz), 6.42 (1H, s), 6.52 (1H, s), 7.10—7.18 (2H, m), 7.53 (1H, dd, J=1.32, 6.92 Hz). FAB-MS *m/z*: 573 (M^++1).

1-[4-(4-Hydroxy-2,3,5-trimethylphenoxy)butyl]-2-[4-[4-(4-hydroxy-2,3,5-trimethylphenoxy)butyl]-1-homopiperazino]-1*H*-benzimidazole (26) **4** (1.4 g, 3.9 mmol) and **13** (1.2 g, 3.9 mmol) were mixed at 120 °C for 10 h. The mixture was chromatographed on silica gel ($CHCl_3$–MeOH, 50 : 1) to afford **26** (1.5 g, 2.4 mmol, 61%) as a pale brown amorphous powder. ^{1}H-NMR δ: 1.64—1.81 (6H, m), 1.96—2.06 (4H, m), 2.10 (3H, s), 2.13 (3H, s), 2.15 (3H, s), 2.16 (3H, s), 2.20 (3H, s), 2.21 (3H, s), 2.53 (2H, t, J=7.26 Hz), 2.68—2.82 (4H, m), 3.57 (4H, t, J=5.61 Hz), 3.86—3.91 (5H, m), 4.08 (2H, t, J=7.26 Hz), 6.46 (1H, s), 6.50 (1H, s), 7.06—7.19 (2H, m), 7.54 (1H, dd, J=1.65, 6.59 Hz). FAB-MS *m/z*: 629 (M^++1).

Synthesis of Benzimidazole Derivative Difumarate Benzimidazole derivatives (**16**—**26**) were dissolved in EtOH. Fumaric acid (2.2 eq) in hot EtOH solution was added. The mixture was cooled, evaporated, washed with hexane and dried to afford the benzimidazole derivative difumarate.

Preparation of Passively Sensitized Rat Peritoneal Cell Exudate[16] Male Wistar rats (Japan SLC Inc.) weighing about 300 g were passively sensitized by intraperitoneal injection of 2 ml physiological saline containing 10 μl rat anti-DNP-BSA serum (titer, 1 : 2000 or greater). After 2 d, rats were exsanguinated and injected intraperitoneally with 15 ml Tyrode solution containing 10 mM HEPES and 0.05% gelatin (pH 7.4). The abdominal region was gently massaged for 1.5 min. The peritoneal cell exudate was collected and recovered by washing the cavity with 15 ml Tyrode solution. Cells were washed 3 times with Tyrode solution by centrifugation (55×***g***) at 4 °C for 8 min and resuspended in a small volume of Tyrode solution. Mast cells were counted after staining with toluidine blue.

Assay of Histamine Release from Sensitized Rat Peritoneal Cell Exudate Produced by Antigen[16] To a Tyrode solution containing 10 mM

HEPES and 0.05% gelatin (pH 7.4) was added 30 μg/ml L-α-phosphatidyl-L-serine and 10^{-5} M test compound, followed by preincubation at 37 °C for 5 min. After adding 10^5 mast cells/ml peritoneal cell exudate to the preincubated solution, the resultant solution was incubated at 37 °C for 15 min. To the incubated solution was added 10 μg protein/ml DNP-BSA and the solution was further incubated at 37 °C for 20 min (final volume 1 ml). After ice-cooling for 10 min, the solution was centrifuged at 4 °C for 10 min (300×***g***). An aliquot (0.5 ml) of the supernatant was used to determine the amount of released histamine after adding 0.5 ml 0.2 N HCl. To the precipitate was added 2 ml 0.1 N HCl and the resultant dispersion was treated at 100 °C for 10 min. After ice-cooling for 10 min, the dispersion was centrifuged at 4 °C for 10 min (780×***g***), an aliquot (1 ml) of supernatant was then used to determine the amount of histamine associated with the cells. Determination of the amount of histamine was carried out according to the method of Shore *et al.*[17] Thus, 0.1 ml 2 N NaOH and 0.05 ml 1% *o*-phthalaldehyde MeOH solution were added to 1 ml test sample, which was then allowed to stand at room temperature for 4 min. After adding 0.1 ml 2 M citrate, the fluorescence of the sample was measured (Ex 356 nm, Em 440 nm) and the rate of histamine release calculated.

Measurement of RBL-1 Cell 5-Lipoxygenase Activity This experiment was carried out as described in a previous report.[18]

Measurement of Fe^{3+}–ADP Induced NADPH-Dependent Lipid Peroxidation in Rat Liver Microsomes This experiment was carried out as described in a previous report.[19]

Measurement of Histamine-Induced Contraction of Isolated Guinea Pig Ileum Male Hartley guinea pigs (Japan SLC Inc.) weighing 300—400 g were sacrificed and the ilea were prepared. Segments (about 1 cm) of ileum were suspended in a Magnus tube (35 °C, 95% O_2/5% CO_2) containing 10 ml Tyrode solution. After 30 min, test compound was added and the solution incubated for 5 min. Histamine was cumulatively added to the tube to obtain a concentration–response curve. Contractile responses were measured using an isotonic transducer (TD-112S, Nihon Kohden Co., Ltd.). pA_2 values for test compounds were calculated by the method of Takayanagi.[20]

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