Design and Syntheses of a Series of Novel Serotonin$_3$ Antagonists

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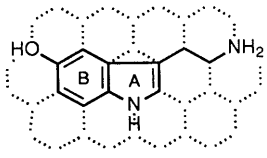
From a structural comparison study between serotonin and serotonin$_3$ (5-HT$_3$) antagonists using a two-dimensional grid template composed of regular hexagons, we deduced structural modification patterns from agonists to antagonists, and designed new 5-HT$_3$ antagonist prototypes. Among them, 2-(4-methyl-1-piperazinyl)-1-butylbenzimidazole (6) was identified as a lead compound which has potent 5-HT$_3$ antagonistic activity comparable to that of granisetron. Using a quantitative structure–activity relationships method, we optimized the structure of 6 and selected 6-amino-5-chloro-1-isopropyl-2-(4-methyl-1-piperazinyl)benzimidazole dimaleate (69, KB-6933), one of the most potent and long-acting 5-HT$_3$ antagonists, as a candidate drug.

Keywords serotonin$_3$ antagonist; benzimidazole derivative; quantitative structure–activity relationship; prototype generation

Serotonin$_3$ (5-HT$_3$) antagonists are used for control of emesis induced by cancer chemotherapeutic agents and are also of interest for treatment of gastrointestinal disturbance and diseases of the central nervous system.[1] Although the existing 5-HT$_3$ antagonists such as ondansetron and granisetron show outstanding efficacy in the control of the emesis over the first 24 h (acute emesis), they do not improve the emesis lasting for several days following cancer chemotherapy (delayed emesis).[2] The reason for this may be weak potency and/or short duration of their 5-HT$_3$ antagonistic activity.

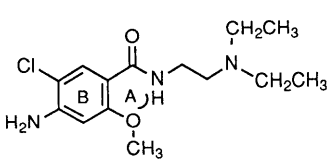
We started an investigation to find potent as well as long-acting 5-HT$_3$ antagonists. There seemed to be no appropriate lead compound among existing 5-HT$_3$ antagonists and almost all known 5-HT$_3$ antagonists have an asymmetric carbon, which may be disadvantageous in preparation. Therefore, we decided not to synthesize analogs of existing 5-HT$_3$ antagonists, but to search for new prototype structures without asymmetric centers. Here, we report the design and syntheses of a new series of 5-HT$_3$ antagonists.

Prototype Generation In order to extract important pharmacophoric elements of antagonists, structural comparison studies are usually performed, often using only structures of antagonists. Such structural comparison studies are useful when antagonists with several skeletons are available. However, usually there are few antagonist prototypes at the start of research. We believe that there should be some structural correspondences between agonists and their antagonists, and if "general" structural conversion patterns from agonists to antagonists can be found, it might be possible to design antagonists from agonist structures, even if there are few prototypes of antagonists. Thus, we have performed a structural comparison study using

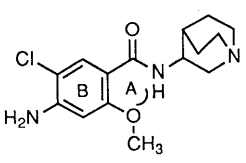


serotonin

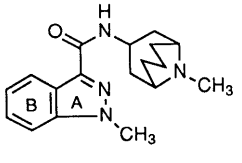
5-HT$_3$ antagonists



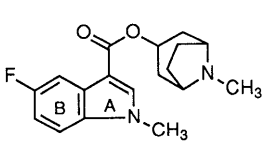
metoclopramide



zacopride



granisetron



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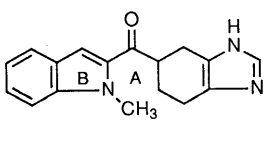
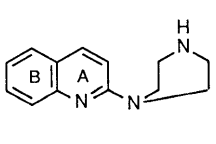
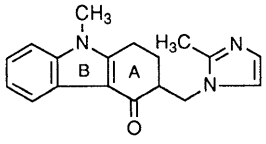


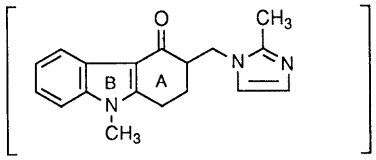
YM-060



quipazine



ondansetron (a)



ondansetron (b)

Chart 1. Structures of Serotonin and Some 5-HT$_3$ Antagonists

structures of both agonists and antagonists of various receptors[3] using a two-dimensional grid template composed of regular hexagons.[4]

The structures of serotonin and 5-HT_3 antagonists are shown in Chart 1. All the antagonists as well as serotonin have an aromatic moiety and a basic amine, and almost all the antagonists have a carbonyl group. First, we superimposed the structure of each 5-HT_3 antagonist on the grid template, fixing the position of the basic amine, so as to overlap each aromatic ring. In this step, a molecule with a rather rigid structure, YM-060, was used as a guide. Then, serotonin was superimposed on the overlapped structures of 5-HT_3 antagonists. In this process, a five-membered ring was drawn using five arbitrary points of the hexagon, and we chose structures giving the maximum overlap of the molecules rather than the most stable conformations of the molecules. The structure of the piperazinyl group of quipazine seems to be unusual. However, when the basic amine was fixed as described above, the piperazinyl group is restricted to the structure as shown on the two-dimensional grid template. In our structural comparison studies, we found good correspondences among dopamine antagonists and histamine antagonists as well as 5-HT_3 antagonists using the structure of piperazine.[3] The grid hexagons on which the indole ring of serotonin is overlaid are assigned as A and B, as shown in Chart 1.

Among the antagonists, the carbonyl groups do not necessarily overlap each other, but the aromatic moiety or a planar region composed of the carbonyl group and the aromatic moiety overlaps serotonin's indole ring. The region corresponding to the indole ring and the basic amine located at two carbons distant from the region seem to be essential for molecules to bind to the 5-HT_3 receptor site. Although serotonin has 1-NH, 5-OH and NH_2 groups which may contribute to hydrogen bonding with the receptor, not all the antagonists have the groups: the basic amine is tertiary substituted and a small group such as a methyl group seemed to be preferred as the substituent; the antagonists have other substituents at positions corresponding to the 1-, 5- and/or 6-positions of the indole ring. The removal of serotonin's 1-NH and 5-OH groups is a common feature of the antagonists, and the introduction of substituents at the basic amine, 1-, 5- and/or 6-position seems to be important for enhancing the potency and/or improving the selectivity. In the 5-HT_3 antagonists, serotonin's 1-NH group overlaps on the methoxy group or carbonyl group. It may be preferable to have a polar element located at the 1-position or its neighborhood.

From the above considerations, we designed a framework for a 5-HT_3 antagonist as shown in Chart 2. We have been interested in the biological activities of piperazine derivatives, and piperazine is a group without any asymmetric carbons, so we selected the piperazinyl group as the basic amine. Since many 5-HT_3 antagonists have a methyl group as a substituent of the basic amine, we also selected the methyl group as a 4-substituent of the piperazinyl group. In order to obtain a planar conformation and to decrease the basicity of the 1-position of the piperazinyl group, we adopted arylic cyclization. Although

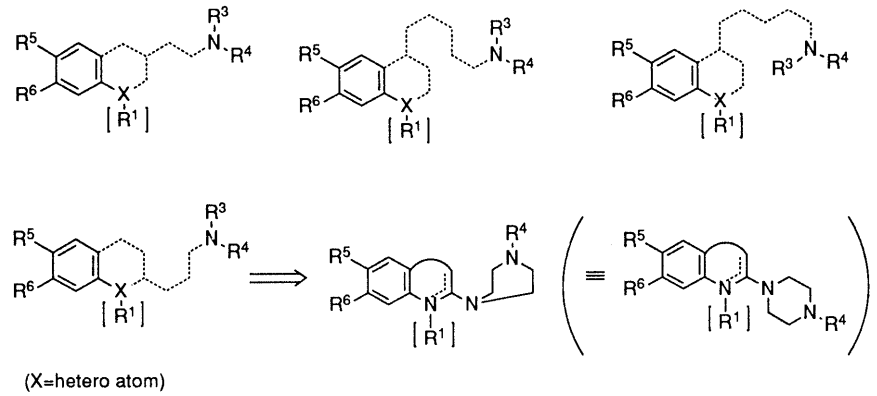


Chart 2. Design of Possible Prototype Structures of 5-HT_3 Antagonists

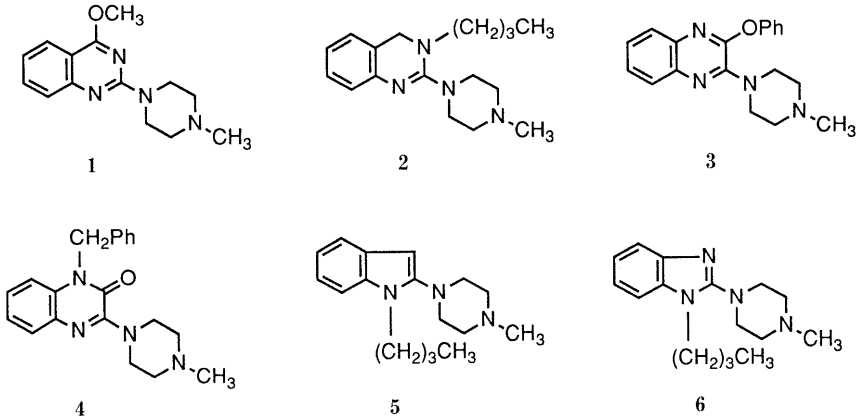


Chart 3. Compounds Which Fit the Designed Prototype

TABLE I. 5-HT_3 Antagonistic Activity of Piperazine Derivatives

Compound No.	Activity[a]	
	100	1
1[b]	++	−
2	++	−
3	++	−
4	++	−
5	NT[d]	−
6[c]	++	+
Ondansetron	++	−
Granisetron	++	++

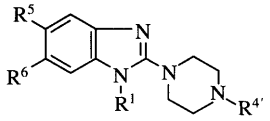
a) Inhibition rate of von Bezold–Jarisch reflex at 100 and 1 μg/kg, i.v.: −, 0—24%; +, 25—49%; ++, 50—100%. *b*) Reference 8. *c*) Reference 7. *d*) Not tested.

quipazine fits the framework, its 5-HT_3 antagonistic activity is not necessarily selective.[5,6]

We have already synthesized several piperazine derivatives which fit the framework (Chart 3), as histamine$_1$ (H_1) antagonistic agents[7] or anticonvulsive agents.[8] We tested their 5-HT_3 antagonistic activity using the von Bezold–Jarisch reflex[9] at 100 μg/kg and 1 μg/kg, i.v., and the results are listed in Table I.

Almost all the 1-arylpiperazine derivatives showed significant 5-HT_3 antagonistic activity (more than 50% inhibition) at 100 μg/kg, as expected. The benzimidazole derivative, **6**, an analog of a potent H_1 antagonist, emedastine,[10] showed the most potent 5-HT_3 antagonistic activity, which is comparable to that of granisetron. When the benzimidazole ring was replaced with an indole ring (**5**),

TABLE II. Structures and 5-HT_3 Antagonistic Activity of Benzimidazole Derivatives



No.	Compound				Activity[a]		Discrimination[b]
	R^1	R^5	R^6	$R^{4'}$	1.0	0.3	
6[c,e]	$(CH_2)_3CH_3$	H	H	CH_3	+		1
7[c]	H	H	H	CH_3	−		1
8[c,e]	CH_3	H	H	CH_3	−		1
9[d]	CH_2CH_3	H	H	CH_3	−		1
10[c,d]	$CH_2CH_2CH_3$	H	H	CH_3	−		1
11[d]	$CH(CH_3)CH_2CH_3$	H	H	CH_3	++	−	2
12[d]	$CH_2CH(CH_3)_2$	H	H	CH_3	−		1
13	$C(CH_3)_3$	H	H	CH_3	−		1
14[c,d]	$(CH_2)_4CH_3$	H	H	CH_3	++	+	2
15[c,d]	$CH(CH_3)CH_2CH_2CH_3$	H	H	CH_3	++	+	2
16[c,d]	$(CH_2)_2CH(CH_3)_2$	H	H	CH_3	−		1
17	Cyclopentyl	H	H	CH_3	++	+	2
18[c,d]	$(CH_2)_5CH_3$	H	H	CH_3	−		1
19[c,d]	$CH_2CH(CH_3)CH_2CH_2CH_3$	H	H	CH_3	−		1
20[c,d]	$(CH_2)_6CH_3$	H	H	CH_3	−		1
21[c,d]	$(CH_2)_9CH_3$	H	H	CH_3	−		1
22[c,e]	CH_2CH_2OH	H	H	CH_3	−		1
23[c,d]	$CH_2CH_2OCH_2CH_3$	H	H	CH_3	−		1
24[c,d]	$(CH_2)_3OCH_3$	H	H	CH_3	−		1
25[e]	Tetrahydrofurfuryl	H	H	CH_3	+		1
26[c]	$CH_2CH_2SCH_2CH_3$	H	H	CH_3	−		1
27[c,e]	$CH_2CH_2NHCH_2CH_3$	H	H	CH_3	−		1
28[c,d]	Ph	H	H	CH_3	−		1
29[c,e]	CH_2Ph	H	H	CH_3	+		1
30[c,d]	CH_2CH_2Ph	H	H	CH_3	−		1
31[c,d]	$(CH_2)_4CH_3$	H	H	H	−		1
32[c,d]	$(CH_2)_4CH_3$	H	H	CH_2CH_3	−		1
33[c,d]	$(CH_2)_4CH_3$	H	H	$CH_2CH_2CH_3$	−		1
34[c,d]	$(CH_2)_4CH_3$	H	H	CH_2Ph	−		1
35[d]	$(CH_2)_3CH_3$	CF_3	H	CH_3	−		1
36[d]	$(CH_2)_3CH_3$	Cl	H	CH_3	++	+	2
37[d]	$(CH_2)_3CH_3$	CH_3	H	CH_3	++	−	2
38[d]	$(CH_2)_3CH_3$	F	H	CH_3	++	−	2
39[d]	$(CH_2)_3CH_3$	OCH_3	H	CH_3	−		1
40	CH_3	Cl	H	CH_3	++	−	2
41	CH_2CH_3	Cl	H	CH_3	++	+	2
42	$CH_2CH_2CH_3$	Cl	H	CH_3	++	+	2
43	$CH(CH_3)CH_2CH_3$	Cl	H	CH_3	++	+	2
44	$CH_2CH(CH_3)_2$	Cl	H	CH_3	+		1
45	Cyclopentyl	Cl	H	CH_3	++	+	2
46	Tetrahydrofurfuryl	Cl	H	CH_3	++	−	2
47[d]	CH_2CH_3	CH_3	H	CH_3	++	−	2
48	$(CH_2)_3CH_3$	H	NO_2	CH_3	−		1
49[d]	$(CH_2)_3CH_3$	H	Cl	CH_3	−		1

TABLE II. (continued)

No.	Compound				Activity[a]		Discrimination[b]
	R^1	R^5	R^6	R^4	1.0	0.3	
50	$(CH_2)_3CH_3$	H	NH_2	CH_3	+		1
51[d]	$(CH_2)_3CH_3$	H	F	CH_3	+		1
52	$(CH_2)_3CH_3$	H	OH	CH_3	+		1
53	CH_3	Cl	NH_2	CH_3	++	++	3
54	CH_2CH_3	Cl	NH_2	CH_3	++	++	3
55	$CH_2CH_2CH_3$	Cl	NH_2	CH_3	++	++	3
56	$(CH_2)_3CH_3$	Cl	NH_2	CH_3	++	++	3
57	$CH(CH_3)CH_2CH_3$	Cl	NH_2	CH_3	++	++	3
58	Cyclopentyl	Cl	NH_2	CH_3	++	++	3
59	Tetrahydrofurfuryl	Cl	NH_2	CH_3	++	++	3
60	CH_2CH_3	CH_3	NH_2	CH_3	++	+	2
61	$(CH_2)_3CH_3$	CH_3	NH_2	CH_3	++	−	2
62	Cyclopentyl	CH_3	NH_2	CH_3	++	−	2
63	$(CH_2)_3CH_3$	F	NH_2	CH_3	++	+	2
64	$(CH_2)_3CH_3$	CH_3	CH_3	CH_3	−		1
65	$(CH_2)_3CH_3$	NO_2	NO_2	CH_3	−		1
66	$(CH_2)_3CH_3$	Cl	NO_2	CH_3	−		1
67	$(CH_2)_3CH_3$	F	NO_2	CH_3	−		1
68	$CH(CH_3)_2$	Cl	H	CH_3	++	++	3
69[e]	$CH(CH_3)_2$	Cl	NH_2	CH_3	++	++	3
70	Cyclopropyl	Cl	H	CH_3	++	+	2
71	Cyclopropyl	Cl	NH_2	CH_3	++	++	3
72[d]	3-Pentyl	Cl	H	CH_3	+		1
73	3-Pentyl	Cl	NH_2	CH_3	+		1
74	$(CH_2)_3OCH_2CH_3$	Cl	H	CH_3	++	−	2
75	$(CH_2)_3OCH_2CH_3$	Cl	NH_2	CH_3	++	++	3

a) Inhibition rate of von Bezold–Jarisch reflex at 1.0 and 0.3 μg/kg, i.v.: −, 0—24%; +, 25—49; ++, 50—100%. *b*) Discrimination: 1 < ++ at 1.0 μg/kg ≦ 2 < ++ at 0.3 μg/kg ≦ 3. *c*) Reference 7. *d*) Fumarate. *e*) Maleate.

the activity disappeared. Based on the above results, we selected compound **6** as a lead for our investigation. Because of the potent H_1 antagonistic activity of **6**,[7] it is necessary in the lead optimization to enhance 5-HT_3 antagonistic activity as well as to improve the selectivity.

Lead Optimization Several benzimidazole derivatives (Table II) were tested for 5-HT_3 antagonistic activity at 1 μg/kg and 0.3 μg/kg, i.v. In order to extract factors for enhancing the activity effectively, we used quantitative structure–activity relationships (QSAR) analyses. The adaptive least-squares technique (ALS)[11] is useful when activity is evaluated at defined doses.

First, we tested benzimidazole derivatives with various substituents at the 1-position and at the piperazine 4-position which had already been prepared, and found that the activity of the 1-substituted derivatives (**6**—**30**) varied according to the substituents, and that among the derivatives with various substituents at the piperazine 4-position (**14**, **31**—**34**), only the methyl analog is active. We classified the 1-substituted derivatives into two discrete groups, those showing significant activity (++) at 1 μg/kg (2) and those not showing such activity (1), and performed the QSAR analysis to obtain Eq. 1.

$$Y = -0.153\pi^2 + 0.789\pi - 0.923D - 0.306 \quad (1)$$

$$n = 25, \quad Rs = 0.702, \quad n_{mis} = 2, \quad t = 4.73 \ (p < 0.001)$$

where Y is the discriminative score for the classification of activity ratings, π is the hydrophobic parameter[12] of the 1-substituent, D is a dummy variable indicating the presence of a branch ($D = 1$) or not ($D = 0$) in the 1-substituent except for the α-position, n represents the number of compounds used to derive the equation, n_{mis} is the number misclassified, Rs is the Spearman's rank correlation coefficient,[11] t is Student's t-value[11] and p is the level of significance. Equation 1 shows that a substituent of suitable hydrophobicity at the 1-position of the benzimidazole ring is favorable and a branch in the 1-substituent except for the α-position was disadvantageous for the activity. The parameters and results of recognition are summarized in Table III.

Next, we prepared benzimidazole derivatives with substituents at 5- and/or 6-positions. Among 1-butyl derivatives with various 5-substituents (**35**—**39**), the potency decrease in the following order; Cl, Me, F > H > OMe, CF_3. QSAR analysis of six derivatives (**6**, **35**—**39**) gave Eq. 2.

$$Y = -2.152(B_4)^2 + 7.243B_4 - 4.872 \quad (2)$$

$$n = 6, \quad Rs = 1, \quad n_{mis} = 0$$

where B_4 is a Verloop's STERIMOL parameter[13] which represents the largest width of the 5-substituents. Equation 2 shows that a substituent of a suitable width such as Cl, Me and F is desirable as the 5-substituent.

The introduction of substituent at the 6-position does not enhance the activity (**48**—**52**), and sterically large groups such as Cl and NO_2 decreased the activity. However, the 5-Cl, 6-NH_2 derivative (**56**) showed more potent activity than the 5-Cl derivative (**36**). QSAR analysis of all derivatives (**6**—**30** and **35**—**67**), which were classified into three discrete groups (3 ≧ ++ at 0.3 μg/kg > 2 ≧ ++ at 1 μg/kg > 1), gave Eq. 3.

$$Y = -0.064\pi^2 + 0.265\pi - 0.534D - 2.351(B_4)^2 + 8.461(B_4)$$

TABLE III. 5-HT_3 Antagonistic Activity and Parameters of Benzimidazole Derivatives

Compd. No.	Parameters				Obsd.	Recognition		Prediction[e]
	π[a]	D[b]	B_4[c]	E_s[d]		Eq. 1	Eq. 3	
6	2.13	0	1.00	0.00	1	2	1	1
7	0.00	0	1.00	0.00	1	1	1	1
8	0.56	0	1.00	0.00	1	1	1	1
9	1.02	0	1.00	0.00	1	1	1	1
10	1.55	0	1.00	0.00	1	1	1	1
11	2.04	0	1.00	0.00	2	1	1	1
12	2.04	1	1.00	0.00	1	1	1	1
13	1.98	0	1.00	0.00	1	1	1	1
14	2.67	0	1.00	0.00	2	2	1	1
15	2.54	0	1.00	0.00	2	2	1	1
16	2.54	1	1.00	0.00	1	1	1	1
17	2.14	0	1.00	0.00	2	2	1	1
18	3.17	0	1.00	0.00	1	1	1	1
19	3.04	0	1.00	0.00	1	1	1	1
20	3.67	0	1.00	0.00	1	1	1	1
21	5.17	0	1.00	0.00	1	1	1	1
22	−0.77	0	1.00	0.00	1	1	1	1
23	0.22	0	1.00	0.00	1	1	1	1
24	0.22	0	1.00	0.00	1	1	1	1
25	0.73	1	1.00	0.00	1	1	1	1
26	1.14	0	1.00	0.00	1	1	1	1
27	−0.11	0	1.00	0.00	1	1	1	1
28	1.96	0	1.00	0.00	1	1	1	1
29	2.01	1	1.00	0.00	1	1	1	1
30	2.66	1	1.00	0.00	1	1	1	1
35	2.13	0	2.61	0.00	1		1	1
36	2.13	0	1.80	0.00	2		2	2
37	2.13	0	2.04	0.00	2		2	2
38	2.13	0	1.35	0.00	2		2	2
39	2.13	0	2.87	0.00	1		1	1
40	0.56	0	1.80	0.00	2		2	2
41	1.02	0	1.80	0.00	2		2	2
42	1.55	0	1.80	0.00	2		2	2
42	2.04	0	1.80	0.00	2		2	2
44	2.04	1	1.80	0.00	1		2	2
45	2.14	0	1.80	0.00	2		2	2
46	0.73	1	1.80	0.00	2		2	2
47	1.02	0	2.04	0.00	2		2	2
48	2.13	0	1.00	−2.52	1		1	1
49	2.13	0	1.00	−0.97	1		1	1
50	2.13	0	1.00	−0.61	1		1	1
51	2.13	0	1.00	−0.46	1		1	1
52	2.13	0	1.00	−0.55	1		1	1
53	0.56	0	1.80	−0.61	3		2	2
54	1.02	0	1.80	−0.61	3		3	2
55	1.55	0	1.80	−0.61	3		3	3
56	2.13	0	1.80	−0.61	3		3	3
57	2.04	0	1.80	−0.61	3		3	3
58	2.14	0	1.80	−0.61	3		3	3
59	0.73	1	1.80	−0.61	3		2	2
60	1.02	0	2.04	−0.61	2		2	2
61	2.13	0	2.04	−0.61	2		2	2
62	2.14	0	2.04	−0.61	2		2	2
63	2.13	0	1.35	−0.61	2		2	2
64	2.13	0	2.04	−1.24	1		2	2
65	2.13	0	2.44	−2.52	1		1	1
66	2.13	0	1.80	−2.52	1		1	2
67	2.13	0	1.35	−2.52	1		1	1
68	1.53	0	1.80	0.00	3			2[f]
69	1.53	0	1.80	−0.61	3			3[f]
70	1.14	0	1.80	0.00	2			2[f]
71	1.14	0	1.80	−0.61	3			3[f]
72	2.54	0	1.80	0.00	1			2[f]
73	2.54	0	1.80	−0.61	1			3[f]
74	0.72	0	1.80	0.00	2			2[f]
75	0.72	0	1.80	−0.61	3			2[f]

a) Hydrophobic parameter of the 1-substituent. *b*) Presence of a branch in the 1-substituent except for α-position, $D=1$. *c*) Verloop's STERIMOL parameter of the 5-substituent. *d*) Taft's steric parameter of the 6-substituent. *e*) Leave-one-out method. *f*) Calculated from Eq. 3.

$$-0.486(Es)^2-0.675(Es)-6.704 \quad (3)$$

$n=58$, $Rs=0.831$, $n_{mis}=8(0)$, $t=11.18$ ($p<0.001$)

where Es means Taft's steric parameter[12] of the 6-substituent. Equation 3 shows that the potency is affected independently by the substituents at the 1-, 5- and 6-positions, and benzimidazole derivatives having a 1-substituent of suitable hydrophobicity without a branch except for the α-position, a 5-substituent of a suitable width and a 6-substituent with a suitable steric effect are expected to show 5-HT_3 antagonistic activity. The 5-Cl, 6-NH_2 groups seem to be suitable substituents. The prediction using the leave-one-out method gave 10 misclassified compounds (Table III), and Rs was calculated to be 0.814, which was statistically significant.

As mentioned above, in order to improve the selectivity of 5-HT_3 antagonistic activity, it is important to remove H_1 antagonistic activity from benzimidazole derivatives. We had already studied the QSAR of the 1-substituted benzimidazole derivatives as H_1 antagonistic agents, obtaining Eq. 4.[14]

$$\log 1/IC_{50}=-0.096\ (\pm 0.025)L^2+1.413(\pm 0.369)L -1.173\ (\pm 0.321)B_3+4.686\ (\pm 1.401) \quad (4)$$

$n=30$, $r=0.891$, $s=0.397$, $F=33.32$

where the number in parentheses is the 95% confidence interval, B_3 means the second largest width parameter and L means a length parameter[13] of the 1-substituent, r is the correlation coefficient, s is the standard deviation and F is the F-ratio between the variances of calculated and observed activities. Equation 4 shows that H_1 antagonistic activity is expected to be reduced by the introduction of a substituent with branching and/or shorter or longer length than the optimum at the 1-position.

On the basis of Eqs. 3 and 4, we synthesized additional derivatives (**69**, **71**, **73** and **75**) which were expected to show potent and selective 5-HT_3 antagonistic activity. Com-

TABLE IV. Comparative Biological Activities of Benzimidazoles and References

Compound No.	Toxicity[a] number of mice died/tested	Anti-5HT$_3$ ID_{50} (μg/kg) i.v. (5)[c]	i.v. (30)[d]	*p.o.*	Anti-H_1[b] IC_{50} (μM)
6					0.019
53	0/5	0.21	2.5		
54	5/5				
55	5/5				
56	5/5				
57	5/5				0.2
58	5/5				
59	0/5	0.18	1.0		
69	0/5	0.071	0.063	0.39	>10
71	5/5				
75	0/5	0.091	0.55		
Ondansetron	(4 mg/kg)[e]	4.0	31		
Granisetron	(25 mg/kg)[f]	0.71	5.3	76	
YM-060	(100 mg/kg)[e]	0.047			

a) Test compound was administered at 100 mg/kg, i.v. *b*) Reference 7. *c*) Tested at 5 min after the drug administration. *d*) Tested at 30 min after the drug administration. *e*) LD_{50} values are from reference 15. *f*) LD_{50} value is from reference 16.

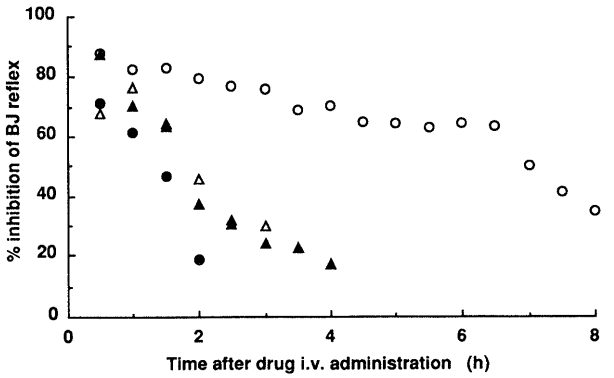


Fig. 1. Time Course of 5-HT3 Antagonistic Activity

○, **69** 0.3; ●, ondansetron 100; △, granisetron 30; ▲, YM-060 0.3 μg/kg i.v.

pounds **69**, **71** and **75** showed potent 5-HT_3 antagonistic activity as expected, but compound **73** did not show activity even at 1 μg/kg.

Evaluation of the 5-HT_3 Antagonists The biological activities of the potent 5-HT_3 antagonistic compounds (**53**—**59**, **69**, **71** and **75**) and reference compounds are summarized in Table IV. When any of **54**—**58** and **71** was given intravenously at a dose of 100 mg/kg to a group of 5 mice, all of the mice died. However, no mice died when **53**, **59**, **69** or **75** was given. These compounds are much safer than ondansetron and granisetron; the LD_{50} values of these compounds in mice were reported to be 4 mg/kg, i.v.[15] and 25 mg/kg, i.v.,[16] respectively. For the low-toxicity compounds and the reference compounds, ID_{50} values of 5-HT_3 antagonistic activity were determined at 5 min after administration. All benzimidazole derivatives (**53**, **59**, **69** and **75**) showed more potent activity than ondansetron and granisetron, and the potency of compound **69** is comparable to that of YM-060, which is one of the most potent 5-HT_3 antagonists known.[17]

In order to study the duration of the efficacy, we determined the ID_{50} values at 30 min after administration, and the ratio between the ID_{50} values tested at 5 min and 30 min after administration was calculated. The low values of the ratio of ondansetron and granisetron (1/8 and 1/7, respectively) confirm that the activity of these reference compounds disappeared rapidly. Although **53**, **59** and **75** did not show any longer duration of action than granisetron, **69** showed an extremely long duration of action. Time courses of 5-HT_3 antagonistic activity at fixed doses of **69** and reference compounds were examined, and the results are shown in Fig. 1. The 5-HT_3 antagonistic activity of ondansetron and granisetron had disappeared by about 2 h after administration when 100 μg/kg, i.v. and 25 μg/kg, i.v. were given, respectively (25—40 times the ID_{50} value). The activity of YM-060 also rapidly disappeared when 0.3 μg/kg, i.v. was given (about 6 times the ID_{50} value). Compound **69** showed significant 5-HT_3 antagonistic activity for about 8 h when 0.3 μg/kg, i.v. was given (4 times the ID_{50} value). We also confirmed 5-HT_3 antagonistic activity of **69** when it was administered *p.o.* The ratio of the ID_{50} values of **69** between i.v. and *p.o.* administration is smaller than that of granisetron, which suggests that the bioavailability of **69** is better than that of granisetron.

Compound **6** showed potent H_1 antagonistic activity, and the 5-Cl, 6-NH_2 analog (**56**) retained the activity, whereas

TABLE V. Binding Assay of **69** to Various Receptors[a]

Receptor	Radioligand	Inhibition (%, $n=2$) 10^{-7}	10^{-5} (M)
Adrenergic α_1	[^{3}H]Prazosin	−11.4	0.1
Adrenergic α_2	[^{3}H]RX 781094	1.6	27.8
Adrenergic β	[^{3}H]DHA	−7.7	−7.2
$Dopamine_1$	[^{3}H]SCH 23390	−1.6	3.7
$Dopamine_2$	[^{3}H]Sulpiride	1.1	2.8
$GABA_A$	[^{3}H]GABA	−3.0	−2.7
$GABA_B$	[^{3}H]GABA + Isoguvacine	−6.6	20.8
$Histamine_1$	[^{3}H]Pyrilamine	7.7	10.4
$Histamine_2$	[^{3}H]Tiotidine	−6.0	10.2
$Serotonin_1$	[^{3}H]5-HT	−6.8	17.1
$Serotonin_{1A}$	[^{3}H]8-OH-DPAT	3.5	−0.7
$Serotonin_{1B}$	[^{125}I]Iodocyanopindolol	−5.4	38.1
$Serotonin_{1C}$	[^{3}H]Mesulergine	13.3	24.4
$Serotonin_2$	[^{3}H]Ketanserin	−0.5	25.2
$Serotonin_3$[b]	[^{3}H]GR-65630	100	100
$Musucarinic_1$	[^{3}H]Pirenzepine	1.9	65.0
$Musucarinic_2$	[^{3}H]AFDX384	1.5	12.8
Nicotinic	[^{3}H]NMCI	−8.6	−2.2
Glutamate	[^{3}H]Glutamate	3.0	4.0
Benzodiazepine	[^{3}H]Flunitrazepam	−1.3	2.6
PCP	[^{3}H]TCP	−1.8	−3.6
Sigma	[^{3}H]DTG	4.9	20.7
Mu	[^{3}H]DAGO	3.3	4.2
Delta	[^{3}H]DPDPEN	2.9	8.8
Kappa	[^{3}H]U69593	3.9	11.6
CCK	[^{125}I]CCK	−1.6	3.3
NPY	[^{125}I]NPY	13.1	−10.2
Somatostatin	[^{125}I]Somatostatin	4.8	0.5
VIP	[^{125}I]VIP	−0.9	8.1
Calcium (Type N)	[^{125}I]Omegaconotoxin	5.9	15.1
Calcium (Type T and L)	[^{3}H]Nitrendipine	−0.7	−3.0
Chloride	[^{3}H]TBOB	5.3	−7.8
Potassium	[^{125}I]Apamin	−0.5	−14.6
Forskolin	[^{3}H]Forskolin	−5.3	−6.9
Phorbol ester	[^{3}H]PDBU	5.5	5.4
Inositol triphosphate	[^{3}H]IP3	2.3	1.1
Dopamine reuptake	[^{3}H]WIN 35428	2.7	9.7
Norepinephrine uptake	[^{3}H]DMI	−1.1	0.2
Serotonin uptake	[^{3}H]Citalopram	8.7	22.7

a) Evaluated by Nova Pharmaceutical Corporation. *b*) $K_i=0.066$ nM, evaluated by Kanebo New Drug Research Laboratories, Reference 18.

69 did not show activity even at 10 μM. In order to evaluate the selectivity of **69** in more detail, receptor binding assays were performed. Compound **69** showed high affinity for the 5-HT_3 receptor site ($K_i=0.066$ nM),[18] and negligible affinity to the other receptor sites (Table V). From the above results, compound **69** is confirmed to be a potent and long-acting 5-HT_3 antagonist. We tested the antiemetic activity of **69**, and found that the potency is higher than that of ondansetron and granisetron, and comparable to that of YM-060[19]; the order of potency is consistent with that of the ID_{50} values of their 5-HT_3 antagonistic activity. We selected **69** (KB-6933) as the most promising candidate for a 5-HT_3 antagonist in this series. Further examinations are continuing.

Discussion

There are several reports on pharmacophores of 5-HT_3 antagonists.[20,21] Schmidt and Peroutka studied the most stable conformation of 5-HT_3 antagonists using computer-aided three-dimensional graphics and reported that the

pharmacophores of antagonists were the planar aromatic moiety, carbonyl group and basic amine.[20] Hibert and co-wokers obtained similar results.[21] These studies seem to validate our use of the two-dimensional molecular model in our study. Our molecular model is useful to find structural differences and similarities between agonists and antagonists without detailed calculations.

Hibert superimposed the structures of the 5-HT_3 antagonists and reported that the aromatic ring, basic amine and carbonyl group of antagonists could occupy the same relative position, and, in the case of ondansetron, the carbon atom centered in N–C–N of the imidazole ring fitted the position of the basic amine when the other elements were superimposed.[21] In our model, a similar result was observed: when the aromatic rings and carbonyl groups of ondansetron and YM-060 were superimposed on each other, the basic amine of YM-060 was overlaid on the carbon atom of N–C–N [see Chart 1, ondansetron (b)]. The low potency of ondansetron may be a result of this structural deviation. However, it remains questionable whether the 5-HT_3 receptor has an interaction site for the carbonyl group, because serotonin and compound **69** do not have the carbonyl group but show strong affinity.

Serotonin, dopamine and histamine are neurotransmitter amines with 2-arylethylamine structure, and their receptors are proposed to be similar.[22] Therefore, it seems reasonable that analogs of dopamine antagonists or histamine antagonists show 5-HT_3 antagonistic activity. Metoclopramide is a potent dopamine antagonist,[23] and granisetron was developed through a lead optimization study of metoclopramide as a 5-HT_3 antagonist.[24] Compound **69** is the first active compound to be found as an analog of an H_1 antagonist.

Chemistry

2-(1-Piperazinyl)benzimidazole (Table II) were synthesized by methods A, B, C and D as shown in Chart 4.

In method A, 2-(4-methyl-1-piperazinyl)benzimidazole[7] was alkylated at the 1-position of the benzimidazole nucleus to afford the desired compounds. Method A is useful for the preparation of 1-substituted benzimidazole derivatives, but can not be used for selective preparation of 1,5- or 1,6-disubstituted benzimidazole derivatives because the alkylation occurs at the 3-position as well as the 1-position of the benzimidazole nucleus. The 1,5- and 1,6-disubstituted 2-(4-methyl-1-piperazinyl)benzimidazole derivatives were prepared by reaction of *N*-methylpiperazine with 1,5- or 1,6-disubstituted 2-chlorobenzimidazoles, which were prepared by reaction of the corresponding 2-benzimidazolones with $POCl_3$ (method B). The 2-benzimidazolones were prepared from *o*-chloronitrobenzenes *via* 3 steps as previously reported by us[7] (Table VII). The 6-OH derivative (**52**) was prepared by hydrogenation of its benzyl analog, which was prepared by method B. Nitration of 2-(4-methyl-

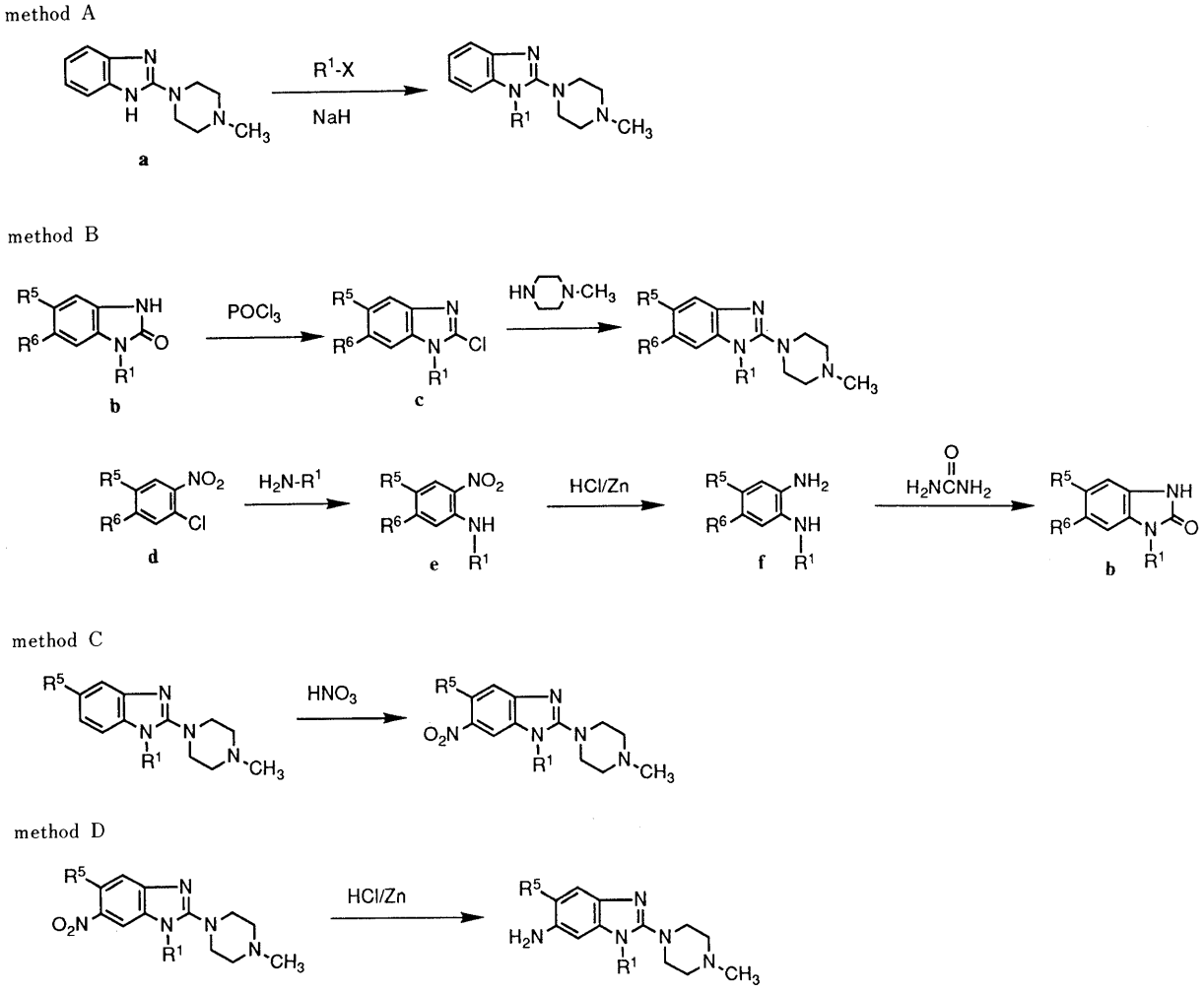


Chart 4. Syntheses of Benzimidazole Derivatives

TABLE VI. 2-(4-Methyl-1-piperazinyl)benzimidazole Derivatives

No.	Compound R[1]	R[5]	R[6]	Method	Yield[a] (%)	mp (°C)	Formula[b]	Analysis (%) Calcd C	Calcd H	Calcd N	Found C	Found H	Found N
9	CH_2CH_3	H	H	A	73	170.5—172.0	$C_{14}H_{20}N_4 \cdot 2FA$	55.46	5.92	11.76	55.28	5.82	11.65
11	$CH(CH_3)CH_2CH_3$	H	H	A	48	163.0—166.0	$C_{16}H_{24}N_4 \cdot 2FA$	57.13	6.39	11.10	57.24	6.47	11.03
12	$CH_2CH(CH_3)_2$	H	H	A	82	183.5—185.0	$C_{16}H_{24}N_4 \cdot 3/2FA$	59.18	6.77	12.55	59.06	6.69	12.67
13	$C(CH_3)_3$	H	H	B	10	125.5—126.0	$C_{16}H_{24}N_4$	70.55	8.88	20.57	70.50	8.74	20.73
17	Cyclopentyl	H	H	B	21	128.5—130.0	$C_{17}H_{24}N_4$	71.79	8.51	19.70	71.65	8.36	19.59
25	Tetrahydrofurfuryl	H	H	A	36	154.5—156.5	$C_{17}H_{24}N_4O \cdot 2MA$	56.38	6.06	10.52	56.56	5.87	10.59
35	$(CH_2)_3CH_3$	CF_3	H	B	39	186.0—194.0	$C_{17}H_{23}ClN_4 \cdot 3/2FA$	53.69	5.68	10.89	53.84	5.63	11.06
36	$(CH_2)_3CH_3$	Cl	H	B	26	167.0—169.0	$C_{16}H_{23}ClN_4 \cdot 3/2FA$	54.94	6.08	11.65	54.82	5.90	11.62
37	$(CH_2)_3CH_3$	CH_3	H	B	35	147.0—162.0	$C_{17}H_{26}N_4 \cdot 3/2FA$	59.99	7.00	12.17	59.83	6.80	12.11
38	$(CH_2)_3CH_3$	F	H	B	39	170.0—172.0	$C_{16}H_{23}FN_4 \cdot 3/2FA$	56.89	6.29	12.06	56.95	6.17	12.13
39	$(CH_2)_3CH_3$	OCH_3	H	B	34	174.0—177.0	$C_{17}H_{26}N_4O \cdot 3/2FA$	57.97	6.77	11.76	57.82	6.69	11.74
40	CH_3	Cl	H	B	53	139.0—141.0	$C_{13}H_{17}ClN_4$	58.98	6.47	21.16	58.90	6.38	21.06
41	CH_2CH_3	Cl	H	B	30	124.0—125.5	$C_{14}H_{19}ClN_4$	60.32	6.87	20.10	60.34	6.87	20.03
42	$CH_2CH_2CH_3$	Cl	H	B	33	89.0—91.0	$C_{15}H_{21}ClN_4$	61.53	7.23	19.13	61.37	7.13	19.26
43	$CH(CH_3)CH_2CH_3$	Cl	H	B	63	104.0—106.0	$C_{16}H_{23}ClN_4$	62.63	7.56	18.26	62.73	7.70	18.37
44	$CH_2CH(CH_3)_2$	Cl	H	B	64	145.0—147.0	$C_{16}H_{23}ClN_4$	62.63	7.56	18.26	62.61	7.70	18.26
45	Cyclopentyl	Cl	H	B	42	168.0—171.0	$C_{17}H_{23}ClN_4$	64.04	7.27	17.57	64.00	7.17	17.75
46	Tetrahydrofurfuryl	Cl	H	B	26	96.0—98.0	$C_{17}H_{23}ClN_4O$	60.98	6.92	16.73	60.87	6.76	16.80
47	CH_2CH_3	CH_3	H	B	37	168.0—171.0	$C_{15}H_{22}N_4 \cdot 2FA$	56.32	6.16	11.42	56.28	6.03	11.56
48	$(CH_2)_3CH_3$	H	NO_2	C	86	192.0—194.0	$C_{16}H_{23}N_5O_2$	55.42	6.28	16.16	55.35	6.17	16.25
49	$(CH_2)_3CH_3$	H	Cl	B	41[c]	180.0—183.0	$C_{16}H_{23}ClN_4 \cdot FA$	56.80	6.44	13.25	56.87	6.53	13.33
50	$(CH_2)_3CH_3$	H	NH_2	D	73	93.0—98.0	$C_{16}H_{25}N_5 \cdot H_2O$	62.97	8.91	22.93	62.86	8.91	22.90
51	$(CH_2)_3CH_3$	H	F	B	50	186.0—194.0	$C_{17}H_{23}FN_4 \cdot FA \cdot 1/4H_2O$	56.34	6.34	11.95	56.42	6.09	12.03
52	$(CH_2)_3CH_3$	H	OH	B	54	163.0—166.0	$C_{16}H_{24}N_4O$	66.64	8.39	19.43	66.54	8.47	19.54
53	CH_3	Cl	NH_2	D	53	181.0—183.0	$C_{13}H_{18}ClN_5$	55.81	6.48	25.03	55.83	6.40	24.97
54	CH_2CH_3	Cl	NH_2	D	67	184.0—187.0	$C_{14}H_{20}ClN_5$	57.23	6.86	23.84	57.36	6.73	23.82
55	$CH_2CH_2CH_3$	Cl	NH_2	D	72	180.5—182.0	$C_{15}H_{22}ClN_5$	58.53	7.20	22.75	58.45	7.07	22.73
56	$(CH_2)_3CH_3$	Cl	NH_2	D	68	160.0—164.0	$C_{16}H_{24}ClN_5$	59.71	7.52	21.76	59.62	7.41	21.73
57	$CH(CH_3)CH_2CH_3$	Cl	NH_2	D	82	207.0—210.0	$C_{16}H_{24}ClN_5$	59.71	7.52	21.76	59.73	7.70	21.75
58	Cyclopentyl	Cl	NH_2	D	13	220.5—222.5	$C_{17}H_{24}ClN_5$	61.16	7.25	20.98	61.06	7.08	21.15
59	Tetrahydrofurfuryl	Cl	NH_2	D	58	163.0—165.0	$C_{17}H_{24}ClN_5O$	58.36	6.91	20.02	58.20	6.88	20.06
60	CH_2CH_3	CH_3	NH_2	D	68	148.0—153.0	$C_{15}H_{23}N_5 \cdot 1/4H_2O$	64.83	8.52	25.20	64.91	8.63	25.24
61	$(CH_2)_3CH_3$	CH_3	NH_2	D	56	155.0—158.0	$C_{17}H_{27}N_5$	67.74	9.03	23.23	67.88	9.21	23.18
62	Cyclopentyl	CH_3	NH_2	D	51	194.0—196.0	$C_{18}H_{27}N_5$	68.97	8.68	22.34	68.90	8.76	22.46
63	$(CH_2)_3CH_3$	F	NH_2	D	41	124.5—125.0	$C_{16}H_{24}FN_5$	62.93	7.92	22.93	62.86	7.71	22.86
64	$(CH_2)_3CH_3$	CH_3	CH_3	B	56	95.5—96.0	$C_{18}H_{28}N_4$	71.96	9.39	18.65	71.97	9.54	18.70
65	$(CH_2)_3CH_3$	NO_2	NO_2	C	34	107.0—109.0	$C_{16}H_{22}N_6O_4$	53.03	6.12	23.19	52.86	5.98	23.17
66	$(CH_2)_3CH_3$	Cl	NO_2	C	70	108.0—110.0	$C_{16}H_{22}ClN_5O_2$	54.62	6.30	19.91	54.60	6.23	19.86
67	$(CH_2)_3CH_3$	F	NO_2	C	50	111.5—112.0	$C_{16}H_{22}FN_5O_2$	57.30	6.61	20.88	57.34	6.42	20.99
68	$CH(CH_3)_2$	Cl	H	B	42	124.0—125.5	$C_{15}H_{21}ClN_4$	61.53	7.23	19.13	61.30	7.40	19.25
69	$CH(CH_3)_2$	Cl	NH_2	D	73	171.0—172.0	$C_{15}H_{22}ClN_5 \cdot 2MA$	51.16	5.60	12.97	51.13	5.58	13.15
70	Cyclopropyl	Cl	H	B	43	105.5—106.5	$C_{15}H_{19}ClN_4$	61.96	6.59	19.27	62.02	6.61	19.18
71	Cyclopropyl	Cl	NH_2	D	52	205.0 (dec.)	$C_{15}H_{20}ClN_5$	58.91	6.59	22.90	58.83	6.49	23.01
72	3-Pentyl	Cl	H	B	68	201.0—202.0	$C_{17}H_{25}ClN_4 \cdot FA$	57.73	6.69	12.82	57.54	6.63	12.76
73	3-Pentyl	Cl	NH_2	D	55	220.0—221.0	$C_{17}H_{26}ClN_5$	60.79	7.80	20.85	60.86	7.95	20.99
74	$(CH_2)_3OCH_2CH_3$	Cl	H	B	37	72.0—74.0	$C_{17}H_{25}ClN_4O \cdot 1/4H_2O$	59.81	7.53	16.41	59.84	7.50	16.47
75	$(CH_2)_3OCH_2CH_3$	Cl	NH_2	C	43[d]	119.0—120.0	$C_{17}H_{26}ClN_5O$	58.03	7.45	19.90	57.92	7.56	19.81
76	CH_3	Cl	NO_2	C	77	132.0—134.0	$C_{13}H_{16}ClN_5O_2$	50.41	5.21	22.61	50.34	5.21	22.79
77	CH_2CH_3	Cl	NO_2	C	82	184.0—187.0	$C_{14}H_{18}ClN_5O_2$	57.23	6.86	23.84	57.36	6.73	23.82
78	$CH_2CH_2CH_3$	Cl	NO_2	C	71	139.0—140.5	$C_{15}H_{20}ClN_5O_2$	53.33	5.97	20.73	53.27	5.86	20.84
79	$CH(CH_3)CH_2CH_3$	Cl	NO_2	C	89	208.0—212.0	$C_{16}H_{22}ClN_5O_2FA$	51.34	5.60	14.97	51.49	5.57	15.07
80	Cyclopentyl	Cl	NO_2	C	89	168.0—170.0	$C_{17}H_{22}ClN_5O_2$	56.12	6.09	19.25	56.32	6.04	19.43
81	Tetrahydrofurfuryl	Cl	NO_2	C	77	125.0—127.0	$C_{16}H_{22}ClN_5O_2$	53.75	5.84	18.44	53.76	5.74	18.43
82	CH_2CH_3	CH_3	NO_2	C	94	197.0—202.0	$C_{15}H_{21}N_5O_2 \cdot FA$	54.41	6.01	16.70	54.24	5.91	16.62
83	$(CH_2)_3CH_3$	CH_3	NO_2	C	24	80.0—85.0	$C_{17}H_{25}N_5O_2$	61.61	7.60	21.14	61.61	7.60	21.14
84	Cyclopentyl	CH_3	H	B	52	125.0—125.5	$C_{18}H_{26}N_4 \cdot 1/10H_2O$	72.01	8.80	18.66	71.91	8.79	18.67
85	$CH(CH_3)_2$	Cl	NO_2	C	58	160.0—161.5	$C_{15}H_{20}ClN_5O_2$	53.33	5.97	20.73	53.21	5.85	20.73
86	Cyclopropyl	Cl	NO_2	C	60	178.5—180.0	$C_{15}H_{18}ClN_5O_2$	53.65	5.40	20.86	53.77	5.42	20.89
87	3-Pentyl	Cl	NO_2	C	56	119.5—120.0	$C_{17}H_{24}ClN_5O_2$	55.81	6.61	19.14	55.70	6.51	19.21

a) Yield from starting material of the indicated methods. *b*) FA, fumaric acid; MA, maleic acid. *c*) Yield from 2,5-dichloronitrobenzene. *d*) Yield from **74**.

1-piperazinyl)benzimidazoles firstly occurred at the 6-position, and secondarily at the 5-position (method C). The nitro group was hydrogenated with HCl–Zn to form an amino group (method D). New 2-(4-methyl-1-piperazinyl)benzimidazole derivatives are listed in Table VI.

Experimental

Melting points were measured with a capillary melting point apparatus

TABLE VII. 2-Benzimidazolone Derivatives

No.	Compound R^1	R^5	R^6	Yield[a] (%)	mp (°C)	Formula	Analysis (%) Calcd C	Calcd H	Calcd N	Found C	Found H	Found N
13b	$C(CH_3)_3$	H	H	58	147.5—148.5	$C_{11}H_{14}N_2O$	69.45	7.42	14.72	69.31	7.26	14.75
17b	Cyclopentyl	H	H	63	122.5—123.0	$C_{12}H_{14}N_2O$	71.26	6.98	13.85	71.14	6.92	14.00
35b	$(CH_2)_3CH_3$	CF_3	H	17	135.0—136.5	$C_{12}H_{13}F_3N_2O$	55.81	5.07	10.85	56.00	5.10	11.10
36b	$(CH_2)_3CH_3$	Cl	H	40	141.0—143.0	$C_{11}H_{13}ClN_2O$	58.80	5.83	12.47	59.00	5.81	12.32
37b	$(CH_2)_3CH_3$	CH_3	H	67	128.0—131.0	$C_{12}H_{16}N_2O$	70.56	7.89	13.71	70.37	7.77	13.56
38b	$(CH_2)_3CH_3$	F	H	20	111.0—112.0	$C_{11}H_{13}FN_2O$	63.45	6.29	13.45	63.54	6.43	13.42
39b	$(CH_2)_3CH_3$	OCH_3	H	42	94.0—97.0	$C_{12}H_{16}N_2O_2$	65.43	7.32	12.72	65.35	7.34	12.69
42b	$CH_2CH_2CH_3$	Cl	H	26	182.0—184.0	$C_{10}H_{11}ClN_2O$	57.02	5.26	13.30	56.86	5.25	13.37
43b	$CH(CH_3)CH_2CH_3$	Cl	H	43	140.0—142.0	$C_{11}H_{13}ClN_2O$	58.80	5.83	12.47	58.72	5.81	12.58
44b	$CH_2CH(CH_3)_2$	Cl	H	74	145.0—153.0	$C_{11}H_{13}ClN_2O$	58.80	5.83	12.47	58.81	5.74	12.47
45b	Cyclopentyl	Cl	H	45	156.0—158.0	$C_{12}H_{13}ClN_2O$	60.89	5.54	11.83	61.15	5.60	12.04
46b	Tetrahydrofurfuryl	Cl	H	49	157.0—158.5	$C_{12}H_{13}ClN_2O_2$	57.04	5.19	11.01	56.95	5.20	11.10
51b	$(CH_2)_3CH_3$	H	F	46	130.5—132.0	$C_{11}H_{13}FN_2O$	63.45	6.29	13.45	63.47	6.38	13.51
68b	$CH(CH_3)_2$	Cl	H	44	183.0—186.5	$C_{10}H_{11}ClN_2O$	57.02	5.26	13.30	57.07	5.32	13.38
70b	Cyclopropyl	Cl	H	14	213.5—214.5	$C_{10}H_9ClN_2O$	57.57	4.35	13.43	57.37	4.51	13.39
72b	3-Pentyl	Cl	H	57	147.0—148.0	$C_{12}H_{15}ClN_2O$	60.38	6.33	11.74	60.31	6.25	11.82
74b	$(CH_2)_3OCH_2CH_3$	Cl	H	21	100.5—102.0	$C_{12}H_{15}ClN_2O_2$	56.59	5.94	11.00	56.80	5.89	11.06
84b	Cyclopentyl	CH_3	H	52	147.0—150.0	$C_{13}H_{16}N_2O_2 \cdot 1/2H_2O$	69.31	7.61	12.43	69.27	7.52	12.41

a) Yield from 2,5-dichloronitrobenzene.

TABLE VIII. Correlation Matrix of Parameters

	π	π^2	D	B_4	$(B_4)^2$	E_s	$(E_s)^2$
π	1.000						
π^2	0.890	1.000					
D	0.028	0.001	1.000				
B_4	−0.037	−0.161	−0.141	1.000			
$(B_4)^2$	−0.016	−0.136	−0.145	0.988	1.000		
E_s	−0.103	0.007	0.186	−0.232	−0.211	1.000	
$(E_s)^2$	0.107	0.024	−0.141	0.157	0.152	−0.947	1.000

(Yamato MP-21) and are uncorrected. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were taken on a Hitachi R-24B NMR spectrometer with tetramethylsilane (TMS) as an internal standard. Chemical shifts are given as δ values (ppm): s, singlet; d, doublet; t, triplet; q, quartet; br s, broad singlet; dd, double doublet; m, multiplet. Elemental analyses were performed by the Analytical Department of Kanebo Research Center. For column chromatography, Merck Silica gel 60 was used. Receptor binding assays were performed by Nova Pharmaceutical Corporation.

2-(4-Methyl-1-piperazinyl)-3-butyl-3,4-dihydroquinazoline Difumarate (2) A mixture of 2-chloro-3,4-dihydroquinazoline[25] (25 g, 0.15 mol), *N*-methylpiperazine (30 g, 0.30 mol) and $CHCl_3$ (100 ml) was stirred at room temperature for 1 h. The reaction mixture was washed successively with 2 N NaOH and water, dried over $MgSO_4$, and concentrated. The residue was recrystallized from CH_3CN to give 2-(4-methyl-1-piperazinyl)-3,4-dihydroquinazoline (19 g) as colorless prisms. mp 123.0—127.0 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.35 (3H, s), 2.45 (4H, t, J=7 Hz), 3.42 (4H, t, J=7 Hz), 4.37 (2H, s), 4.52 (1H, br s), 6.7—7.3 (4H). *Anal.* Calcd for $C_{13}H_{18}N_4$: C, 67.80; H, 7.88; N, 24.33. Found: C, 67.86; H, 7.78; N, 24.42. A mixture of 2-(4-methyl-1-piperazinyl)-3,4-dihydroquinazoline (2.3 g, 10 mmol), 1-bromobutane (1.4 g, 10 mmol), sodium hydride (NaH, 60% in oil, 0.48 g, 12 mmol) and *N*,*N*-dimethylformamide (DMF, 25 ml) was stirred at room temperature for 5 h. The reaction mixture was diluted with water, and extracted with AcOEt. The extract was washed with water, dried over $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography with $CHCl_3$–MeOH (2:1) to give 2-(4-methyl-1-piperazinyl)-3-butyl-3,4-dihydroquinazoline (1.0 g, 36%) as a yellow oil. ^{1}H-NMR ($CDCl_3$) δ: 0.82 (3H, t, J=6 Hz), 1.0—1.7 (4H), 2.30 (3H, s), 2.43 (4H, t, J=6 Hz), 3.10 (2H, t, J=7 Hz), 3.40 (4H, t, J=6 Hz), 4.11 (2H, s), 6.8—7.3 (4H). 2-(4-Methyl-1-piperazinyl)-3-butyl-3,4-dihydroquinazoline was treated with fumaric acid (0.8 g) to give **2** as colorless leaflets. mp 177.0—180.0 °C(dec.). *Anal.* Calcd for $C_{17}H_{26}N_4 \cdot 2C_4H_4O_4$: C, 57.91; H, 6.61; N, 10.80. Found: C, 57.86; H, 6.55; N, 10.78.

2-(4-Methyl-1-piperazinyl)-4-phenoxyquinoxaline (3) A mixture of 2-chloro-3-(4-methyl-1-piperazinyl)quinoxaline[26] (5.2 g, 20 mmol), phenol (2.3 g, 24 mmol), NaH (1.2 g, 30 mmol) and DMF (40 ml) was stirred at room temperature for 2 h. The reaction mixture was diluted with water, and extracted with $CHCl_3$. The extract was washed with water, dried over $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography with AcOEt–MeOH (10:1), and recrystallized from hexane to give **3** (4.0 g, 62%) as yellowish prisms. mp 95.0—96.0 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.40 (3H, s), 2.70 (4H, t, J=6 Hz), 3.87 (4H, t, J=6 Hz), 6.8—7.7 (4H), 8.05 (5H). *Anal.* Calcd for $C_{19}H_{20}N_4O$: C, 71.23; H, 6.29; N, 17.49. Found: C, 71.22; H, 6.25; N, 17.48.

1-Benzyl-3-(4-methyl-1-piperazinyl)quinoxalin-2(1*H*)-one (4) A mixture of 3-(4-methyl-1-piperazinyl)quinoxalin-2(1*H*)-one[26] (4.9 g, 20 mmol), benzyl bromide (4.1 g, 24 mmol), NaH (1.2 g, 30 mmol) and DMF (40 ml) was stirred at 60 °C for 1 h. The reaction mixture was diluted with water, and extracted with $CHCl_3$. The extract was washed with water, dried over $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography with AcOEt–MeOH (10:1), and recrystallized from 50% EtOH to give **4** (2.2 g, 33%) as yellowish prisms. mp 108.0—109.0 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.35 (3H, s), 2.58 (4H, t, J=6 Hz), 4.02 (4H, t, J=6 Hz), 5.45 (2H, s), 7.0—7.7 (9H). *Anal.* Calcd for $C_{20}H_{22}N_4O$: C, 71.83; H, 6.63; N, 16.75. Found: C, 71.69; H, 6.53; N, 16.65.

1-Butyl-2-(4-methyl-1-piperazinyl)indole Fumarate (5) A mixture of 3-ethoxycarbonyl-2-(4-methyl-1-piperazinyl)indole[27] (3.2 g, 11 mmol), 1-bromobutane (1.8 g, 13 mmol), NaH (0.9 g, 22 mmol) and DMF (40 ml) was stirred at 60 °C for 4 h. The reaction mixture was diluted with water, and extracted with AcOEt. The extract was washed with water, dried over $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography with $CHCl_3$–MeOH (5:1) to give 1-butyl-3-ethoxycarbonyl-2-(4-methyl-1-piperazinyl)indole (1.4 g) as a brown oil. ^{1}H-NMR ($CDCl_3$) δ: 0.97 (3H, t, J=7 Hz), 1.1—1.9 (4H), 1.4 (3H, t, J=7 Hz), 2.35 (3H, s), 2.55 (4H, t, J=6 Hz), 3.35 (4H, t, J=6 Hz), 4.16 (2H, t, J=7 Hz), 4.43 (2H, q, J=7 Hz), 7.0—8.2 (4H). 1-Butyl-3-ethoxycarbonyl-2-(4-methyl-1-piperazinyl)indole was treated with oxalic acid to give its oxalate as brown prisms, mp 191.0—194.0 °C. *Anal.* Calcd for $C_{20}H_{29}N_3O_2 \cdot C_2H_2O_4$: C, 60.95; H, 7.21; N, 9.69. Found: C, 60.73, H, 7.25; N, 9.53. 1-Butyl-3-ethoxycarbonyl-2-(4-methyl-1-piperazinyl)indole oxalate (1.4 g, 2.7 mmol) was dissolved in concentrated HCl (8 ml), and refluxed for 30 min. The solution was neutralized with 2 N NaOH, and extracted with AcOEt. The extract was washed with water, dried over $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography with $CHCl_3$–MeOH (20:1), and treated with fumaric acid to give **5** (0.48 g, 46%) as colorless prisms. mp 159.0—161.0 °C. ^{1}H-NMR (DMSO-d_6) δ: 0.90 (3H, t, J=6 Hz), 1.0—2.0 (4H), 2.40 (3H, s), 2.6—3.2 (8H), 3.96 (2H, t, J=7 Hz), 5.89 (1H, s), 6.57 (2H, s), 6.8—7.5 (4H), 10.12 (2H, s). *Anal.* Calcd for $C_{17}H_{25}N_3 \cdot C_4H_4O_4$: C, 65.10; H,

7.54; N, 10.85. Found: C, 65.07; H, 7.37; N, 10.86.

1-(2-Butyl)-2-(4-methyl-1-piperazinyl)benzimidazole Difumarate (11) (Method A) A mixture of 2-(4-methyl-1-piperazinyl)benzimidazole[7] (**a**, 5.0 g, 23 mmol), 2-chlorobutane (12 g, 130 mmol), NaH (2.2 g, 55 mmol) and DMF (50 ml) was stirred at 80 °C for 20 h. The reaction mixture was diluted with water, and extracted with $CHCl_3$. The extract was washed with water, dried over $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography with $CHCl_3$–MeOH (10 : 1) to give 1-(2-butyl)-2-(4-methyl-1-piperazinyl)benzimidazole (2.6 g). ^{1}H-NMR ($CDCl_3$) δ: 0.75 (3H, t, J=7 Hz), 1.65 (3H, d, J=7 Hz), 1.8—2.2 (2H, m), 2.38 (3H, s), 2.60 (4H, t, J=7 Hz), 3.32 (4H, t, J=7 Hz), 4.0—4.6 (1H, m), 6.9—7.7 (4H). 1-(2-Butyl)-2-(4-methyl-1-piperazinyl)benzimidazole was treated with fumaric acid to give **11**.

1-Butyl-5-chloro-2-(4-methyl-1-piperazinyl)benzimidazole Sesquifumarate (36) (Method B) A mixture of 1-butyl-5-chloro-2-benzimidazolone (**36b**, 5.0 g, 22 mmol) and $POCl_3$ (7 ml) was refluxed for 2 h. The reaction mixture was poured onto ice-water, neutralized with 3 N NaOH, and extracted with $CHCl_3$. The extract was washed with water, dried over $MgSO_4$, and concentrated to give 1-butyl-2,5-dichlorobenzimidazole (**36c**, 4.5 g). ^{1}H-NMR ($CDCl_3$) δ: 0.95 (3H, t, J=7 Hz), 1.2—2.0 (4H), 4.15 (2H, t, J=7 Hz), 7.2—7.65 (3H). A mixture of **36c** (3.0 g), *N*-methylpiperazine (5.0 g, 50 mmol) and xylene (5 ml) was refluxed for 3 h. The reaction mixture was diluted with AcOEt, washed with water, dried over $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography with $CHCl_3$–MeOH (10 : 1) to give 1-butyl-5-chloro-2-(4-methyl-1-piperazinyl)benzimidazole (1.2 g) as an oil. ^{1}H-NMR ($CDCl_3$) δ: 0.95 (3H, t, J=7 Hz), 1.2—2.2 (4H), 2.43 (3H, s), 2.65 (4H, t, J=7 Hz), 3.30 (4H, t, J=7 Hz), 4.00 (2H, t, J=7 Hz), 7.2—7.6 (3H). 1-Butyl-5-chloro-2-(4-methyl-1-piperazinyl)benzimidazole was treated with fumaric acid to give **36**.

1-Butyl-5-chloro-2-(4-methyl-1-piperazinyl)-6-nitrobenzimidazole (66) (Method C) Fuming HNO_3 (3.0 g, 72 mmol) was added dropwise to a mixture of **36** (2.0 g, 6.5 mmol) and acetic acid (5 ml) at 0 °C, and the mixture was stirred at 50 °C for 1 h. The reaction mixture was poured onto ice-water, neutralized with 2 N NaOH, and extracted with AcOEt. The extract was washed with water, dried over $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography with $CHCl_3$–MeOH (6 : 1) to give **66** (1.6 g). ^{1}H-NMR ($CDCl_3$) δ: 0.96 (3H, t, J=7 Hz), 1.2—2.1 (4H), 2.38 (3H, s), 2.63 (4H, t, J=7 Hz), 3.48 (4H, t, J=7 Hz), 4.06 (2H, t, J=7 Hz), 7.71 (1H, s), 7.97 (1H, s).

6-Amino-1-butyl-5-chloro-2-(4-methyl-1-piperazinyl)benzimidazole (56) (Method D) Zn powder (6.0 g, 92 mmol) was added portionwise to a mixture of **66** (5.0 g, 14 mmol), concentrated HCl (10 ml) and EtOH (30 ml) at 60 °C, and the mixture was stirred at 60 °C for 3 h. The reaction mixture was basified with 28% NH_4OH, and extracted with AcOEt. The extract was washed with water, dried over $MgSO_4$, and concentrated. The residue was purified by silica gel column chromatography with $CHCl_3$–MeOH (6 : 1) to give **56** (3.1 g). ^{1}H-NMR ($CDCl_3$) δ : 0.95 (3H, t, J=7 Hz), 1.2—2.1 (4H), 2.37 (3H, s), 2.60 (4H, t, J=7 Hz), 3.30 (4H, t, J=7 Hz), 3.92 (2H, t, J=7 Hz), 4.03 (2H, br s), 6.68 (1H, s), 7.55 (1H, s).

1-Butyl-5-Chloro-2-benzimidazolone (36b) A mixture of 2,5-dichloronitrobenzene (30 g, 160 mmol) and 1-butylamine (50 g, 680 mmol) was refluxed for 3.5 h. The reaction mixture was diluted with AcOEt, washed with water, dried over $MgSO_4$, and concentrated to give *N*-butyl-4-chloro-2-nitroaniline (**36e**, 36 g) as an orange oil. ^{1}H-NMR ($CDCl_3$) δ: 0.95 (3H, t, J=7 Hz), 1.2—1.8 (4H), 3.25 (2H, m), 6.26 (1H, d, J=9 Hz), 7.31 (1H, dd, J=9, 3 Hz), 7.95 (2H, br s), 8.10 (1H, d, J=3 Hz). *Anal.* Calcd for $C_{10}H_{13}N_2O_2Cl$: C, 52.52; H, 5.73; N, 12.25. Found: C, 52.48; H, 5.75; N, 12.23. Zn powder (34 g, 520 mmol) was added portionwise to a mixture of **36e** (34 g, 150 mmol), 2.5 N NaOH (30 ml) and EtOH (100 ml) under gentle reflux, and the mixture was refluxed for 30 min. The reaction mixture was diluted with AcOEt (200 ml), and filtered. The filtrate was washed with water, dried over $MgSO_4$, and concentrated to give 2-amino-*N*-butyl-4-chloroaniline (**36f**, 33 g). ^{1}H-NMR ($CDCl_3$) δ: 0.95 (3H, t, J=7 Hz), 1.1—1.8 (4H), 3.04 (2H, t, J=7 Hz), 3.20 (3H, bs), 6.49 (1H, d, J=8 Hz), 6.65 (1H, dd, J=8, 3 Hz), 6.75 (1H, d, J=3 Hz). *Anal.* Calcd for $C_{10}H_{15}N_2Cl$: C, 60.45; H, 6.61; N, 14.10. Found: C, 60.50; H, 7.74; N, 13.89. A mixture of **36f** (31 g) and urea (25 g, 420 mmol) was stirred at 160 °C for 4 h. The reaction mixture was diluted with AcOEt, washed successively with 1 N HCl, saturated $NaHCO_3$ and water, dried over $MgSO_4$, and concentrated. The residue was recrystallized from isopropyl ether to give **36b** (14 g) as light pink needles. ^{1}H-NMR ($CDCl_3$) δ: 0.91 (3H, t, J=7 Hz), 1.1—1.9 (4H), 3.80 (2H, t, J=7 Hz), 6.8—7.2 (3H), 10.75 (1H, br s).

5-HT$_3$ Antagonistic Activity: Serotonin-Induced Activation of von Bezold-Jarisch Reflex in Urethane-Anesthetized Rats Male Sprague–Dawley rats (210–345 g, Charles River) were anesthetized with urethane (1.25 mg/kg, i.p.), a tracheotomy was performed, and an endotracheal tube was inserted (SP-120). The carotid artery was cannulated and connected to a pressure transducer (MPU-0.5-2900-0-III, Nihon Koden). A femoral vein was exposed, cannulated with SP-45, and used for i.v. drug administration. Heart rate and blood pressure were monitored with the use of the pressure transducer signal and a cardiotachometer coupler (AT-641G, Nihon Koden).

For i.v. evaluation of 5-HT$_3$ antagonists, an initial response to 5-HT was generated. When 5-HT-induced bradycardia returned to control levels, either antagonist or saline was administered, and 5-HT-induced bradycardia was elicited again 5 or 30 min after antagonist or saline administration. For oral studies, conscious rats were dosed (1 ml/kg) with either antagonist or vehicle 1 h before the 5-HT challenge, then the rats were anesthetized with urethane and surgically prepared as indicated above.

H$_1$ Antagonistic Activity: Contraction of Isolated Ileum from Guinea Pigs Induced by Histamine Segments (1 cm) of ileum isolated from guinea pigs were suspended in an organ bath containing Tyrode solution (ventilation, 32 °C). The contractile responses to histamine (5.4×10^{-7} mol/l) were measured with an isotonic transducer (TD-112S, Nihon Koden). Each test compound was added to the organ bath 5 min before the administration of histamine.

References and Notes

1) F. D. King, G. J. Sanger, *Drugs Future*, **14**, 875 (1989).
2) M. S. Aapro, *Drugs*, **42**, 551 (1991).
3) The result will be reported elsewhere.
4) H. Yoshioka, "Rational Approaches to Structure, Activity, and Ecotoxicology of Agrochemicals," ed. by W. Draber, T. Fujita, CRC Press Boca Raton Florida, 1992, pp. 185—217.
5) R. A. Glennon, R. M. Slusher, R. A. Lyon, M. Titeler, J. D. McKenney, *J. Med. Chem.*, **29**, 2375 (1986).
6) R. D. Glennon, A. E. M. Ismaiel, B. G. McCarthy, S. J. Peroutka, *Eur. J. Pharmacol.*, **168**, 387 (1989).
7) R. Iemura, T. Kawashima, T. Fukuda, K. Ito, G. Tsukamoto, *J. Med. Chem.*, **29**, 1178 (1986).
8) M. Hori, R. Iemura, H. Hara, A. Ozaki, T. Sukamoto, H. Ohtaka, *Chem. Pharm. Bull.*, **38**, 681 (1990).
9) J. R. Fozard, M. Host, *Br. J. Pharmacol.*, **77**, 520p (1982).
10) T. Fukuda, Y. Morimoto, R. Iemura, T. Kawashima, G. Tsukamoto, K. Ito, *Arzneim.-Forsch./Drug Res.*, **34** (II), 801 (1984).
11) I. Moriguchi, K. Komatsu, Y. Matsushita, *J. Med. Chem.*, **23**, 20 (1980).
12) C. Hansch, A. Leo, "Substituent Constants For Correlation Analysis in Chemistry and Biology" John Wiley & Sons, Inc., New York, 1979.
13) A. Verloop, W. Hoogenstraten, J. Tipker, "Drug Design," Vol. VII. ed. by E. J. Ariens, Academic Press Inc., New York, 1976, pp. 165—207.
14) R. Iemura, H. Ohtaka, *Chem. Pharm. Bull.*, **37**, 967 (1989).
15) S. Hakkansson, J. A. Artus, A. S. Kelvin, M. Abdi, Y. Nishioka, M. Iwasaki, *Kiso To Rinsyo*, **24**, 4991 (1990).
16) Annual Drug Data Report, 1990, p. 869.
17) K. Miyata, T. Kamato, M. Yamano, A. Nishida, H. Ito, Y. Katsuyama, H. Yuki, R. Tsutsumi, M. Ohta, M. Takeda, K. Honda, *J. Pharmacol. Exp. Ther.*, **259**, 815 (1991).
18) A. Ozaki, R. Maki, Y. Fujishima, T. Sukamoto, *Jpn. J. Pharmacol.*, **61S**, 71 (1993).
19) Unpublished results.
20) A. W. Schmidt, S. J. Peroutka, *Mol. Pharmacol.*, **36**, 505 (1989).
21) M. F. Hibert, R. Hoffmann, R. C. Miller, A. A. Carr, *J. Med. Chem.*, **33**, 1594 (1990).
22) M. F. Hibert, S. T.-Kallmeyer, A. Bruinvels, J. Hoflack, *Mol. Pharmacol.*, **40**, 8 (1990).
23) R. A. Harrington, C. W. Hamilton, R. N. Brogden, J. A. Linkwich, J. A. Romankiewicz, R. C Heel, *Drugs*, **24**, 451 (1983).
24) J. Bermudez, C. S. Fake, G. F. Joiner, K. A. Joiner, F. D. King, W. D. Miner, G. J. Sanger, *J. Med. Chem.*, **33**, 1924 (1990).
25) F. Ishikawa, J. Saegusa, K. Inamura, K. Sakuma, S. Ashida, *J. Med. Chem.*, **28**, 1387 (1985).
26) E. L. Engelhard, W. C. Lumma, Jr., W. S. Saari, Ger. Patent 2433397 (1975) [*Chem. Abstr.*, **82**, 156377 (1933)]
27) J. Bourdais, A. Deberly, Fr. Patent 2131826 (1973) [*Chem. Abstr.*, **78**, 136061*p* (19734)].