Chem. Pharm. Bull. **43**(8) 1364—1378 (1995) Vol. 43, No. 8

Development of Potent Serotonin-3 (5-HT_3) Receptor Antagonists. I. Structure–Activity Relationships of 2-Alkoxy-4-amino-5-chlorobenzamide Derivatives

Hiroshi HARADA, Toshiya MORIE, Yoshimi HIROKAWA, Naoyuki YOSHIDA, and Shiro KATO*

Exploratory Research Laboratories, Dainippon Pharmaceutical Co., Ltd., Enoki 33–94, Suita, Osaka 564, Japan.
Received January 27, 1995; accepted March 25, 1995

A new series of 2-alkoxy-4-amino-5-chlorobenzamide derivatives bearing five- to seven-membered heteroalicyclic rings in the amine moiety was synthesized and evaluated for serotonin-3 (5-HT_3) receptor antagonistic activity by assaying the ability to antagonize the von Bezold–Jarisch reflex in rats. The five- to seven-membered heteroalicycles comprise pyrrolidine, morpholine, 1,4-thiazine, piperidine, piperazine, 1,4-oxazepine, 1,4-thiazepine, azepine, and 1,4-diazepine rings. Among them, some benzamide derivatives having a 1,4-diazepine ring showed a potent 5-HT_3 receptor antagonistic activity. In particular, 4-amino-5-chloro-*N*-(1,4-dimethylhexahydro-1*H*-1,4-diazepin-6-yl)-2-ethoxybenzamide (96) and the 1-benzyl-4-methylhexahydro-1*H*-1,4-diazepine analogue 103 showed potent 5-HT_3 receptor antagonistic activity without 5-HT_4 receptor binding affinity.

Key words serotonin-3 receptor antagonist; von Bezold–Jarisch reflex; 1*H*-1,4-diazepine; mosapride; serotonin-4 receptor agonist

Nausea and emesis induced by anticancer agents and radiation treatment are common side effects that can cause patients to refuse subsequent chemotherapeutic sessions.[1] The clinical effectiveness of various traditional antiemetics such as dopamine D_2 receptor antagonists (domperidone,[2] various phenothiazines *etc.*), metoclopramide,[3] dexamethasone, tetrahydrocannabinoids, and various combinations thereof[4,5] has been evaluated. From these studies, high-dose intravenous metoclopramide emerged as the single most effective agent against cisplatin-induced nausea and emesis. However, metoclopramide often causes side effects such as extrapyramidal symptoms, which are a consequence of its dopamine D_2 receptor antagonistic activity[3] and hence restrict its usefulness.[6–9] Metoclopramide is a relatively weak serotonin-3 (5-HT_3) receptor antagonist[10] as well as a dopamine D_2 receptor antagonist. It has been shown that at least four subtypes of serotonin receptor exist,[11] of which the most important in the emetic process is the 5-HT_3 receptor subtype.[12–14] That finding paved the way to the development of potent and selective 5-HT_3 receptor antagonists.

The Beecham group showed that selectivity of action could be achieved by restricting the conformational freedom of the (diethylamino)ethyl side chain of metoclopramide. The search for selective 5-HT_3 receptor antagonists has resulted in the identification of a number of compounds. In particular, granisetron (BRL 43694) was identified as a particularly potent and highly selective 5-HT_3 receptor antagonist.[15–17] Granisetron has already been used clinically as an antiemetic.

Concurrently, a number of potent and selective 5-HT_3 receptor antagonists have been developed [tropisetron (ICS 205-930),[18] ondansetron (GR 38032F),[19] zacopride,[20] *etc.*] and shown to be effective in the control of cancer chemotherapy-induced emesis.[21] Moreover, the 5-HT_3 receptor antagonists may represent important drugs; recent data have suggested the existence of 5-HT_3 receptor binding sites in the brain[22] and several 5-HT_3 receptor antagonists are currently being evaluated in clinics as antischizophrenic, antimigrainic, and anxiolytic agents[23–26] and as drugs for gastrointestinal dysfunction, such as irritable bowel syndrome.[27]

We have previously reported the synthesis of 4-amino-5-chloro-2-ethoxy-*N*-{[4-(4-fluorobenzyl)-2-morpholinyl]methyl}benzamide (mosapride), which showed a potent gastroprokinetic activity without dopamine D_2 receptor antagonistic activity.[28] Mosapride is a partial agonist at a new serotonin receptor subtype (5-HT_4), a property which has been correlated with gastroprokinetic activity. Mosapride, on the other hand, has not only a potent 5-HT_4 receptor agonistic activity but also a relatively weak 5-HT_3 receptor antagonistic activity; its 5-HT_3 receptor

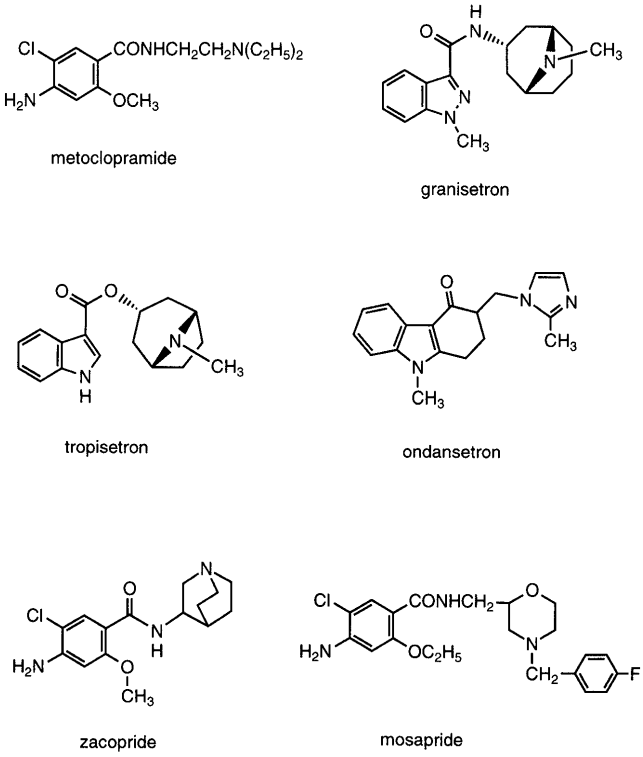


Fig. 1

* To whom correspondence should be addressed.

Table 1. Effect on the B-J Reflex in Rats

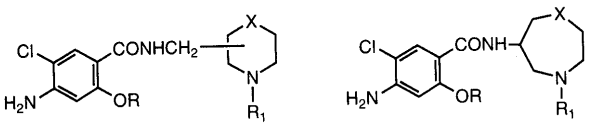
Compd.	ED_{50} (95% C.L.) (μg/kg, i.v.)
Mosapride	261 (71.5—956.4)
Granisetron[a]	0.26 (0.07—0.91)
Ondansetron[a]	1.10 (0.35—3.27)
Metoclopramide	224 (52.1—965.1)
Tropisetron[a]	0.39 (0.11—1.34)

a) See ref. 55.

Table 2. Protection against Cisplatin-Induced Emesis in Ferrets

Compd.	mg/kg, i.v. ×2[a]	Protection[b]	Latency to emetic episodes (min) Mean±S.E.	Number of emetic episodes Mean±S.E.
Saline		0/4	65.8± 4.3	34.5±3.4
Mosapride[c]	1.0	0/4	79.0± 5.1	19.0±4.5[d]
	3.0	0/4	90.0± 6.6[d]	16.0±4.1[d]
	6.0	1/4	113.0±24.0	6.3±2.8[e]
Metoclopramide	1.0	1/4	107.3±24.7	12.0±5.6[d]
	3.0	2/4	145.0±20.6	4.3±2.5[e]

a) Treatment schedule: first dose 30 min before, followed by second dose, 45 min after cisplatin. *b*) Number of ferrets completely protected/ferrets used. *c*) Mosapride was used as citric acid salt. The superscripts *d* are *e* indicate a statistically significant difference from the saline control (Williams–Wilcoxon's multiple test). *d*) $p<0.05$. *e*) $p<0.01$.



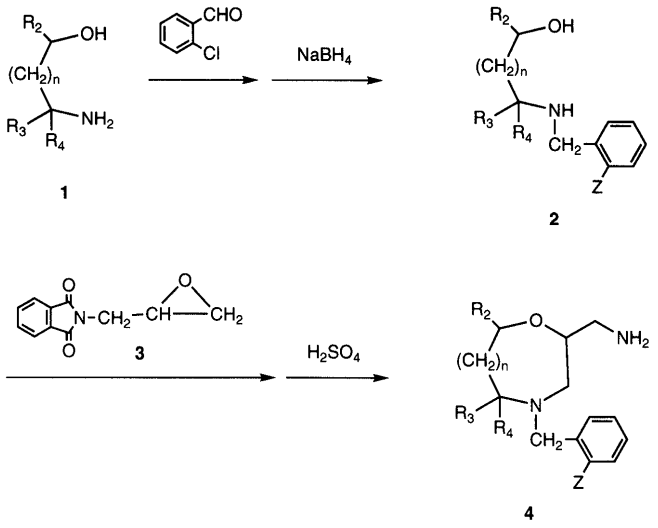
R_1 = CH_2Ph, CH_3, C_2H_5, *etc.*

R = CH_3, C_2H_5, *etc.*

X = —, O, S, CH_2, NCH_3, *etc.*

Fig. 2

antagonistic activity on 2-methyl-5-HT-induced bradycardia (von Bezold–Jarisch reflex; B–J reflex) in rats and its affinity for 5-HT_3 receptor ([^{3}H]quipazine) are ED_{50}= 261 μg/kg, i.v. [*vs.* granisetron; ED_{50}=0.26 μg/kg, i.v. and ondansetron; ED_{50}=1.10 μg/kg, i.v.] (Table 1) and IC_{50}=1380 nM [*vs.* granisetron; IC_{50}=2.0 nM and ondansetron; IC_{50}=4.2 nM] (Table 9), respectively. Furthermore, a high dose (6.0 mg/kg, i.v.) of mosapride, like metoclopramide (3.0 mg/kg, i.v.), considerably inhibited the emetic episodes induced by cisplatin in ferrets (Table 2). To obtain much more potent 5-HT_3 receptor antagonists than mosapride and metoclopramide, a series of 2-alkoxy-4-amino-5-chlorobenzamides bearing five- to seven-membered heteroalicyclic rings in the amine moiety (Fig. 2) and related compounds were prepared. In the present paper, we describe the synthesis of 2-alkoxy-4-amino-5-chlorobenzamide derivatives (**48**—**129**) and evaluation of their structure–activity relationships concerning their 5-HT_3 receptor antagonistic activity.



a: R_2 = CH_3, R_3 = R_4 = H, Z = Cl, n = 0

b: R_2 = R_3 = Z = H, R_4 = CH_3, n = 0

c: R_2 = H, R_3 = R_4 = CH_3, Z = Cl, n = 0

d: R_2 = R_3 = Z = H, R_4 = Ph, n = 0

e: R_2 = R_3 = R_4 = Z = H, n = 1

Chart 1

Chemistry

The requisite amino and acetylamino compounds having a six- or seven-membered ring (**4a**—**e**, **8**, **13**, **15a**, **b**, **16a**, **b**, **20**, **23**, **29**, and **32a**—**c**) were prepared by the methods shown in Charts 1—5. The 5- or 6-substituted and 5,5-dimethyl-2-(aminomethyl)morpholines (**4a**—**d**) and 2-(aminomethyl)-4-benzylhexahydro-1*H*-1,4-oxazepine (**4e**) were synthesized from the corresponding available aminoalcohol derivatives **1a**, **c** and **2b**, **d**, **e** in a similar manner to that described in our previous paper;[29,30] the treatment of 1-amino-2-propanol (**1a**) and 2-amino-2-methyl-1-propanol (**1c**) with 2-chlorobenzaldehyde, followed by reduction with sodium borohydride gave 1-[(2-chlorobenzyl)amino]-2- (**2a**) and 2-[(2-chlorobenzyl)amino]-2-methyl-1-propanols (**2c**), respectively. The aminoalcohols **2a**—**e** were allowed to react with *N*-(2,3-epoxypropyl)phthalimide (**3**), followed by cyclization of the intermediate diols with concentrated sulfuric acid, giving the desired amines **4a**—**e** (Chart 1).

The synthesis of the 5-oxomorpholine derivative **8** was achieved as follows; the reaction of **3** with benzylamine without any solvent and treatment of the resultant aminopropanol **5** with chloroacetyl chloride in the presence of Et_3N afforded the *N*-acetyl propanol **6**. Compound **6** was cyclized with NaH to give the 5-oxomorpholine **7**, which on treatment with hydrazine produced the amine **8**. The phthalimido group of compound **12**[31] was converted to an amino group by treatment with hydrazine, giving 2-(aminomethyl)-1,4-dimethylpiperazine (**13**) (Chart 2).

Our previous paper[32] reported that the nucleophilic reaction of 1-benzyl-2-(chloromethyl)-4-methylpiperazine with NaN_3 in acetonitrile gave a mixture of the ring-expanded 6-azido-1,4-diazepine derivative and the normally substituted piperazine analogue *via* the postulated aziridinium cation intermediate. After the reduction

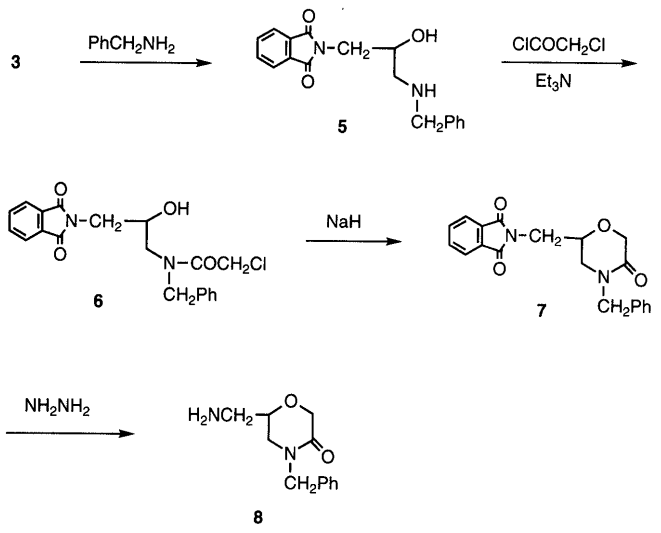


Chart 2

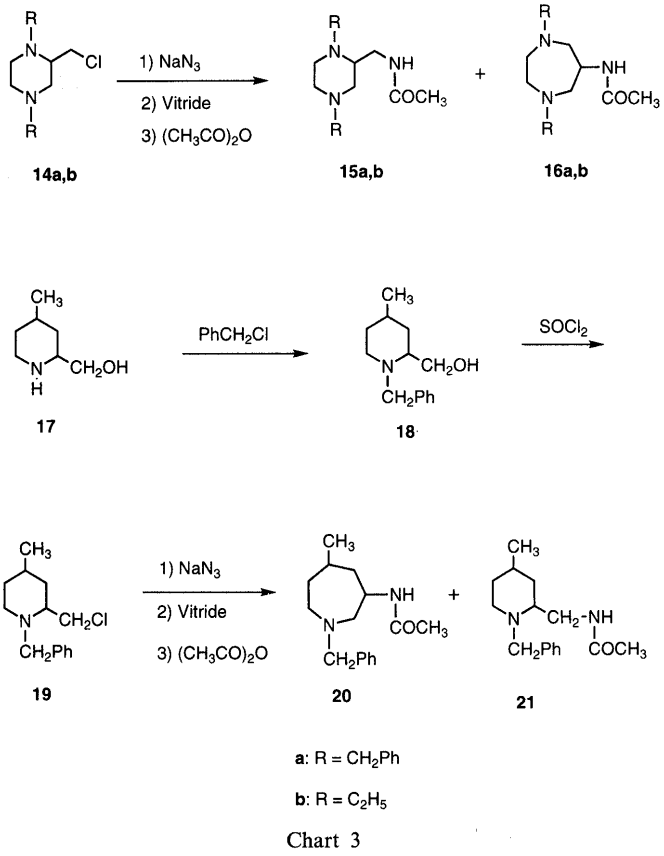


Chart 3

of the azido group with sodium bis(2-methoxyethoxy)-aluminum hydride (Vitride®), followed by acetylation of the resultant 6-amino group with acetic anhydride (Ac_2O), the products were separated into the less polar 6-(acetylamino)-1-benzyl-4-methylhexahydro-1*H*-1,4-diazepine (24%) and the more polar 2-[(acetylamino)-methyl]-1-benzyl-4-methylpiperazine (57%) by medium-pressure column chromatography on silica gel. The structure of each product was confirmed on the basis of the MS and ^{1}H-NMR spectra and alternative synthesis. In the analogous reaction of 1,4-dibenzyl- (**14a**)[33] and 1,4-diethyl-2-(chloromethyl)piperazines (**14b**),[34] the concomitant formation of the ring-expanded seven-membered products (**16a, b**) along with the normally six-membered products (**15a, b**) was observed by TLC and in the ^{1}H-NMR spectrum. The mixture of **15a** and **16a** was separated into the more polar piperazine **15a** (41%) and the less polar 1,4-diazepine **16a** (16%) by medium-pressure column chromatography on silica gel. On the other hand, the ratio of the more polar **15b** and the less polar **16b** (**15b**:**16b**=8.7:1) was determined from the ^{1}H-NMR spectrum. Compounds **15b** and **16b** were derived to the corresponding benzamides **83**/**101** and **84**/**102**, and each product was separated by medium-pressure column chromatography. The structures of compounds **15a**, **16a**, **83**, **84**, **101**, and **102** were consistent with the MS and ^{1}H-NMR data.

1-Benzyl-2-(chloromethyl)-4-methylpiperidine (**19**) was obtained by *N*-benzylation of 2-(hydroxymethyl)-4-methylpiperidine (**17**)[35] with benzyl chloride, followed by the reaction of the product **18** with thionyl chloride. The ring expansion reaction of **19** with NaN_3, followed by reduction of the azido derivatives with Vitride and subsequent acetylation with acetic anhydride afforded a mixture of the less polar azepine **20** and the more polar piperidine **21**. Compounds **20** and **21** were separated by medium-pressure column chromatography in 81 and 16% yields, respectively, and the structures of these compounds were supported by the MS and ^{1}H-NMR data. As reported previously,[32] in the case of the piperazine ring (**14a, b**) the six-membered compound predominated over the seven-membered compound. Conversely, when the ring was piperidine (**17**), the seven-membered product predominated over the six-membered product (Chart 3).

6-Amino-1,4,6-trimethylhexahydro-1*H*-1,4-diazepine (**23**) was obtained by hydrogenation of the known 6-nitro compound **22**[36] in the presence of Raney Ni.

We previously reported the facile synthesis of 6-(acetylamino)-1-benzyl-4-methylhexahydro-1*H*-1,4-diazepine (**24a**) from tris(hydroxymethyl)nitromethane (**25**) and *N*-benzyl-*N'*-methylethylenediamine *via* the corresponding 6-nitro derivative.[31] This method was applied for the preparation of the 6-(acetylamino)-1-benzyl-4-ethylhexahydro-1*H*-1,4-diazepine (**29**). The similar reaction of *N*-benzyl-*N'*-ethylethylenediamine (**26**)[34] with **25** gave the 6-(hydroxymethyl)-6-nitro-1,4-diazepine **27**, which was treated with potassium *tert*-butoxide followed by neutralization with hydroxylamine hydrochloride to produce the 6-nitro-1,4-diazepine **28** as an unstable oil. Compound **28** was immediately hydrogenated in the presence of Raney Ni, followed by acetylation with Ac_2O to produce the desired 1,4-diazepine **29** in a moderate yield (Chart 4).

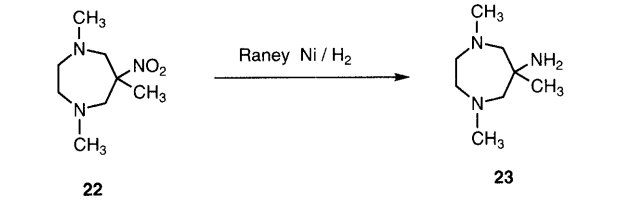
Compounds **32a**—**c** having a fluoro atom on the benzene ring were obtained as shown in Chart 5. Thus, hydrogenolysis of **24a** over palladium on carbon gave the

debenzylated 1,4-diazepine **31**, which was treated with an appropriate fluorobenzyl chloride to afford the 1,4-diazepines **32a—c**. The acetylamino derivatives (**10c**, **15a, b**, **16a, b**, **20**, **29**, **32a—c**) thus prepared were transformed by acidic hydrolysis into the corresponding amines, which were used in the next step.

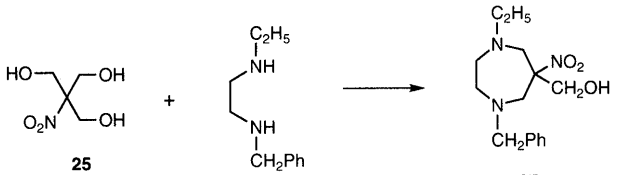
The reaction of 1-(benzylamino)-3-chloro-2-propanol (**34**)[37] with excess methylamine gave the corresponding 1,3-diamine derivative **35**. Formation of 1-benzyl-4-methylhexahydro-1*H*-1,4-diazepin-6-ol (**36**) was successfully achieved by reductive alkylation of **35** with glyoxal in the presence of hydrogen and platinum catalyst (Chart 6).

The known benzoic acid derivatives **40a—l** were obtained from commercial suppliers or prepared according to the literature. 4-Amino-5-chloro-2-propoxybenzoic acids (**41a, b**) were prepared as follows; the treatment of methyl 4-(acetylamino)-2-hydroxybenzoate (**37**)[28a] with *n*- or iso-propyl iodide, and chlorination of the resultant 2-propoxybenzoates **38a, b** with *N*-chlorosuccinimide (NCS), followed by alkaline hydrolysis of the esters **39a, b** gave the benzoic acids **41a, b**, respectively, in good yields (Chart 7).

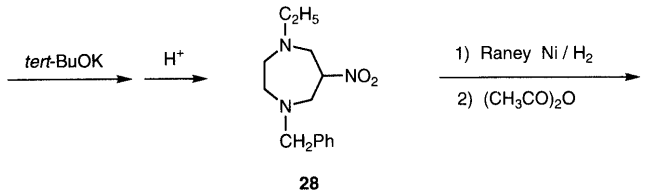
Various benzamides except for compounds **64**, **88**, **115**, and **119** were synthesized by the reaction of the benzoic acid derivatives **40a—l** and **41a, b** with an appropriate amine in the presence of 1-ethyl-3-[3-(dimethylamino)-



22 **23**



25 **26** **27**



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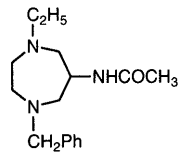

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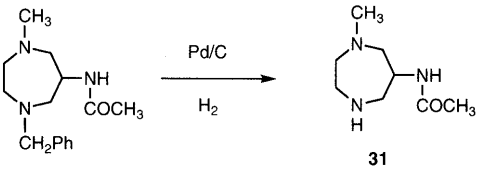
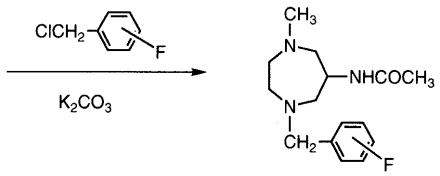
Chart 4



24a **31**



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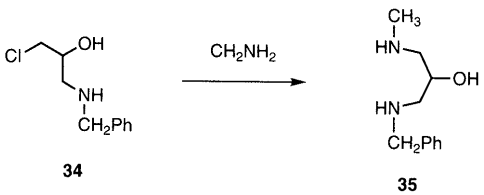
a: 2-F

b: 3-F

c: 4-F

Chart 5



34 **35**

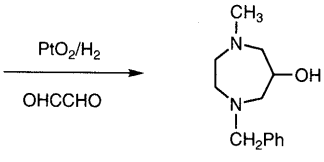


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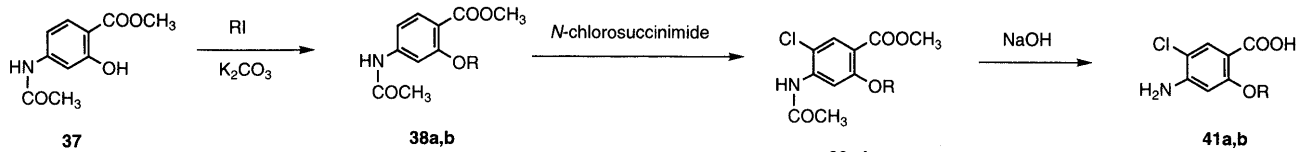
Chart 6



37 **38a,b** **39a,b** **41a,b**

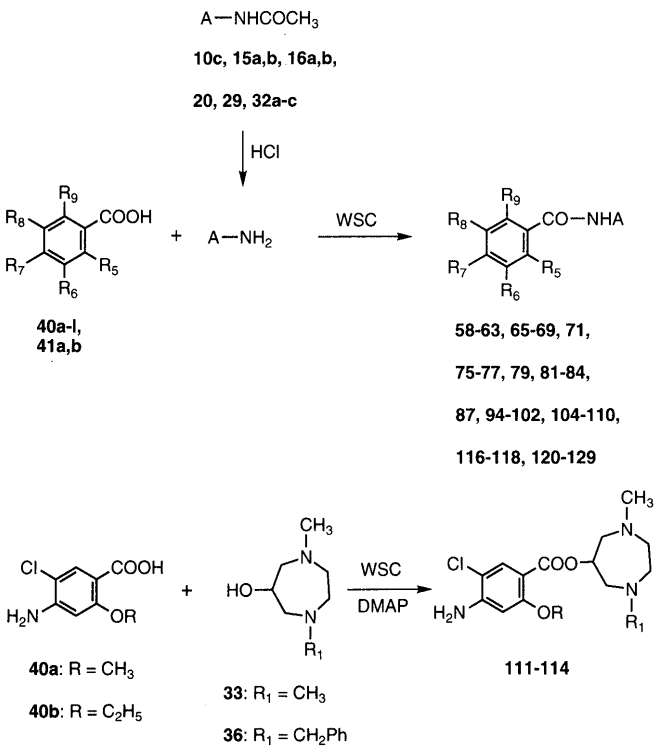
a: R = *n*-C_3H_7

b: R = *iso*-C_3H_7

Chart 7

propyl]carbodiimide hydrochloride (WSC) as a coupling reagent. The esters **111**—**114** were obtained by the treatment of 2-alkoxy-4-amino-5-chlorobenzoic acids (**40a, b**) with the 1,4-diazepin-6-ol **33** or **36** in the presence of WSC and 4-dimethylaminopyridine (DMAP) (Chart 8).

The stereochemistry with respect to the methylene moiety at the 2 position and the methyl group at the 5 position in the morpholine ring of **59** and **60** was determined as follows. The $C_{5\text{-}H}$ coupling constants of **59** and **60** were found to be 13.8/2.9 and 10.3/3.3 Hz, respectively. The data indicate that $C_{5\text{-}H}$ is axially oriented. The coupling constant of $C_{3\text{-}H_2}$ of **60** was observed to be 10.1 and 2.1 Hz with $C_{2\text{-}H}$, so that $C_{2\text{-}H}$ was supposed to be axial. On the other hand, $C_{3\text{-}Heq}$ and $C_{3\text{-}Hax}$ signals of **59** are observed at 2.43 and 2.48 ppm as a doublet of doublets (J=3.8/11.8, 8.1/11.8 Hz), respectively. Since the vicinal coupling constant $J_{3\text{-}Hax-2H}$ of **60** (10.1 Hz) is larger than that of **59** (8.1 Hz), the relative stereochemistry of **59** and **60** is proposed to be *cis* and *trans*, respectively.



A; five- to seven-membered heteroalicylic rings

WSC; 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride

DMAP; 4-dimethylaminopyridine

Chart 8

The 1,4-diazepinyl-2-hydroxybenzamide **43** was prepared from the corresponding 2-methoxybenzamide **104** using the method described in our previous paper.[38] The reaction of the 2-hydroxybenzamides **42** and **43**[29] with $COCl_2$ furnished the 1,3-benzoxazine-2,4-diones **64** and **119**, respectively (Chart 9).

The sulfoxide **88** was obtained by oxidation of **87** with *m*-chloroperbenzoic acid (*m*-CPBA). The acetamide derivative **115**, reversal of the amide linkage of **104**, was prepared from 5-chloro-2-methoxyaniline (**44**); the treatment of **44** with the 1,4-diazepine-6-carboxylic acid dihydrochloride **45**[39] in the presence of WSC afforded the acetamide **46**. Compound **46** was nitrated with a mixed acid (fuming nitric acid and concentrated sulfuric acid) to give the 4-nitro derivative **47**, and subsequent reduction of **47** with stannous chloride in concentrated HCl produced the desired acetamide **115** (Chart 10).

Biological Results and Discussion

Compounds **48**—**129** were evaluated for 5-HT_3 receptor antagonistic activity *in vivo* by measuring their ability to inhibit the B–J reflex induced by 2-methyl-5-HT in rats. The effect is the result of reflex stimulation of the vagus nerve following activation of 5-HT_3 receptors located in the wall of the right ventricle.[40] The results relatively high dose (100 μg/kg, i.v.) are shown in Tables 3—7, and ED_{50} values are shown for the compounds with potent activity. For comparison, ED_{50} values of the reference compounds are shown in Table 1.

The influence of the five- to seven-membered heteroalicycles containing at least one nitrogen atom in the amine moiety on the B–J reflex was first examined. The 2-morpholinyl benzamides prepared previously (**48**—**57**, Table 3) were inactive at this screening dose, like mosapride. Modification of the morpholine ring, *i.e.*, introduction of a methyl or phenyl group and formation of a 5-oxomorpholine or a 1,4-benzoxazine-2,4-dione ring (**58**—**65**, Table 3) failed to enhance the activity. In order to find new benzamides with potent activity, a number of 2-methoxy- and 2-ethoxy-4-amino-5-chlorobenzamides

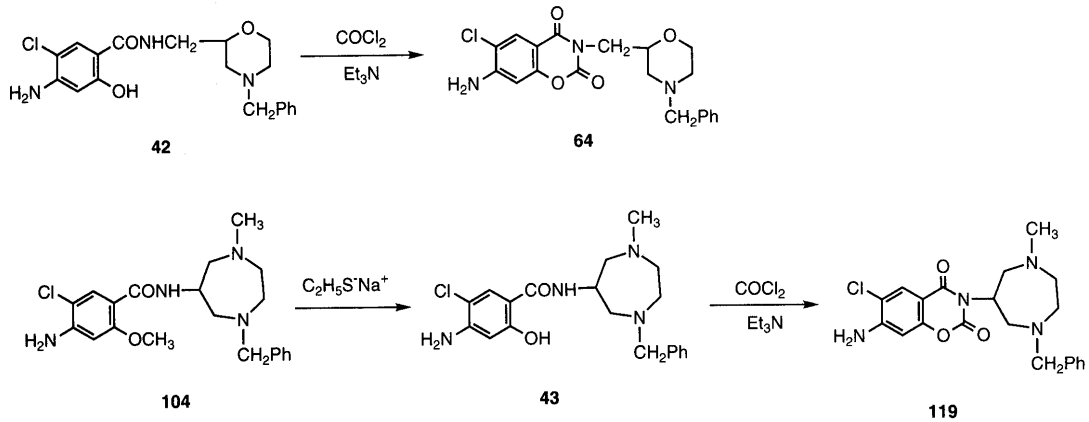
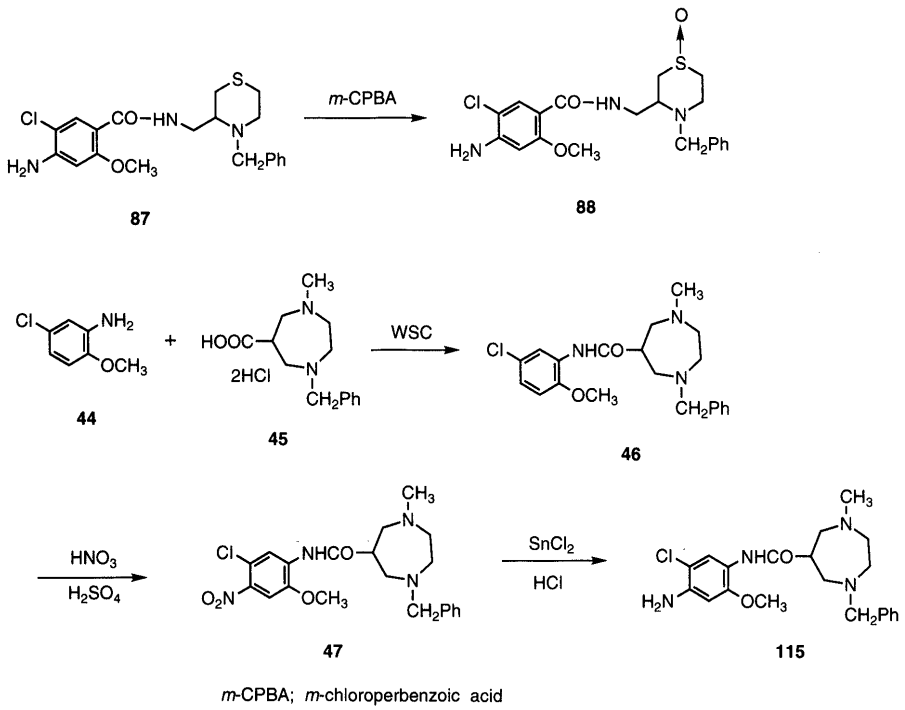


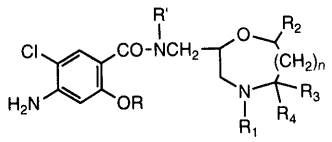
Chart 9



m-CPBA; *m*-chloroperbenzoic acid

Chart 10

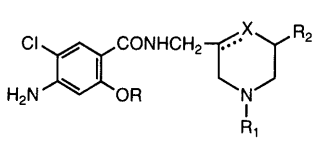
Table 3. Physical Data and Serotonin-3 Receptor Antagonistic Activity for 2-Morpholinyl Benzamides (**48**—**65**)



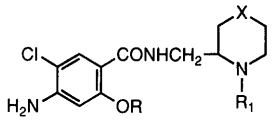
Compd.	R	R′	R_1	R_2	R_3	R_4	*n*	mp (°C) (Recryst. solvent)	Yield[a] (%)	Formula	Analysis (%) Calcd (Found) C	H	N	Cl	Inhibition of B–J reflex[b] (%) 100 μg/kg, i.v.
Mosapride	C_2H_5	H	4-$FC_6H_4CH_2$	H	H	H	0								0
48[c]	C_2H_5	H	$PhCH_2$	H	H	H	0								0
49[d]	C_2H_5	H	3-Pyridylmethyl	H	H	H	0								0
50[d]	CH_3	H	2-Thienylmethyl	H	H	H	0								0
51[d]	C_2H_5	H	$CH_3(CH_2)_5$	H	H	H	0								0
52[d]	C_2H_5	H	$Ph(CH_2)_4$	H	H	H	0								0
53[e]	$(CH_2)_4CH_3$	H	$PhCH_2$	H	H	H	0								0
54[e]	$CH_2CH=CH_2$	H	$PhCH_2$	H	H	H	0								0
55[e]	$CH_2C\equiv CH$	H	$PhCH_2$	H	H	H	0								0
56[e]	CH_2CO_2Et	H	$PhCH_2$	H	H	H	0								0
57[e]	CH_2CN	H	$PhCH_2$	H	H	H	0								0
58	C_2H_5	H	2-$ClC_6H_4CH_2$	CH_3[f]	H	H	0	150—154 (iso-PrOH)	65	$C_{22}H_{27}Cl_2N_3O_3 \cdot 2C_4H_4O_4$[g]	52.64 (52.58)	5.15 (5.27)	6.14 (6.10)	10.36 (10.53)	0
59	C_2H_5	H	$PhCH_2$	H	H	CH_3[h]	0	114—116 (iso-PrOH)	[b]	$C_{22}H_{28}ClN_3O_3 \cdot 2C_4H_4O_4$[g]	58.48 (58.50)	6.04 (6.21)	7.87 (7.69)	6.64 (6.55)	0
60	C_2H_5	H	$PhCH_2$	H	H	CH_3[i]	0	109—111 (iso-PrOH)	[b]	$C_{22}H_{28}ClN_3O_3$	63.23 (62.98	6.75 6.79	10.05 9.78	8.48 8.81)	0
61	C_2H_5	H	2-$ClC_6H_4CH_2$	H	CH_3	CH_3	0	181—184 (EtOH)	76	$C_{23}H_{29}Cl_2N_3O_3$	59.23 (59.33	6.27 6.50	9.01 9.04	15.20 15.07)	0
62	C_2H_5	H	$PhCH_2$	H	H	Ph[f]	0	216—221 (EtOH)	54	$C_{27}H_{30}ClN_3O_3 \cdot 0.75C_4H_4O_4$[g]	63.54 (63.54	5.87 5.77	7.41 7.32	6.23 6.39)	0
63	C_2H_5	H	$PhCH_2$	H	H	H	1	180—183 (iso-PrOH)	61	$C_{22}H_{28}ClN_3O_3 \cdot C_4H_4O_4$[g]	58.48 (58.23	6.04 6.15	7.87 7.61	6.64 6.59)	0
64	CO		$PhCH_2$	H	H	H	0	228—230 ($CHCl_3$–EtOH)	[b]	$C_{20}H_{20}ClN_3O_4 \cdot 0.05CHCl_3$[j]	59.05 (58.88	4.96 4.95	10.30 10.34	10.00 10.23)	0
65	C_2H_5	H	$PhCH_2$	H	O		0	168—170 (MeOH)	34	$C_{21}H_{24}ClN_3O_4$	60.36 (60.32	5.79 5.71	10.06 9.93	8.48 8.52)	0

a) Yields are given for the amine condensation and were not optimized. *b*) See Experimental. *c*) See ref. 29. *d*) See ref. 56. *e*) See ref. 38. *f*) Diastereomeric mixture. *g*) Fumaric acid. *h*) The relative stereochemistry is *cis*. *i*) The relative stereochemistry is *trans*. *j*) The presence of $CHCl_3$ is shown by the ^{1}H-NMR spectrum.

Table 4. Physical Data and Serotonin-3 Receptor Antagonistic Activity for 2-Alkoxy-4-amino-5-chlorobenzamides (**66**—**90**)



66—**84**



85—**90**

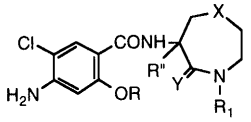
Compd.	R	X	R_1	R_2	mp (°C) (Recryst. solvent[a])	Yield[b] (%)	Formula	Analysis (%) Calcd (Found) C	H	N	Cl	Inhibition of B–J reflex[c] (%) 100 μg/kg, i.v.
66	C_2H_5	—	$PhCH_2$	H	182—185 (M–W)	60	$C_{21}H_{26}ClN_3O_2 \cdot C_2H_2O_4$[d] $\cdot 0.25H_2O$	57.26 (56.99)	5.95 5.89	8.71 8.55	7.35 7.66)	0
67	C_2H_5	—	$PhCH_2$	CH_3[e]	114—117 (E)	68	$C_{22}H_{28}ClN_3O_2 \cdot 1.5C_4H_4O_4$[f]	58.38 (58.41)	5.95 6.20	7.29 7.38	6.15 6.31)	0
68	CH_3	—	$PhCH_2$	CH_3[g]	187—190 (M)	80	$C_{21}H_{26}ClN_3O_2 \cdot 0.25H_2O$	63.49 (63.66	6.53 6.40	11.11 11.01	9.37 9.58)	0
69	C_2H_5	—	$PhCH_2$	C_2H_5[e]	146—149 (I)	59	$C_{23}H_{30}ClN_3O_2 \cdot 1.5C_4H_4O_4$[f]	59.03 (59.14	6.15 6.22	7.12 7.04	6.01 6.19)	0
70[h]	C_2H_5	S	$PhCH_2$	H								30
71	CH_3	S	$PhCH_2$	H	132—134 (M–DE)	58	$C_{20}H_{24}ClN_3O_2S \cdot 0.25H_2O$	58.53 (58.79	6.02 5.96	10.24 10.24	8.64 8.78)	0
72[h]	C_2H_5	SO	CH_2Ph	H								0
73[h]	C_2H_5	SO_2	CH_2Ph	H								0
74[h]	C_2H_5	CH_2	CH_2Ph	H								0
75	CH_3	CH_2	CH_2Ph	H	104—107 (E)	60	$C_{21}H_{27}ClN_3O_2 \cdot 1.5C_2H_2O_4$[d] $\cdot H_2O$	53.29 (53.12	5.78 6.03	7.77 7.61	6.50 6.77)	0
76	C_2H_5	CH	CH_2Ph	H	144—147 (E–T)	88	$C_{22}H_{26}ClN_3O_2$	66.07 (65.87	6.55 6.39	10.51 10.55	8.86 9.00)	22
77	CH_3	CH	CH_2Ph	H	147—150 (E–T)	85	$C_{21}H_{24}ClN_3O_2$	65.36 (65.56	6.27 6.28	10.89 10.78	9.19 9.22)	0
78[h]	C_2H_5	NCH_2Ph	CH_2Ph	H								0
79	CH_3	NCH_2Ph	CH_2Ph	H	103—110 (E–DE)	75	$C_{27}H_{31}ClN_3O_2 \cdot C_2H_2O_4$[d] $\cdot H_2O$	59.33 (59.34	6.01 5.89	9.54 9.29	6.04 5.79)	0
80[h]	C_2H_5	NCH_3	CH_2Ph	H								41
81	C_2H_5	NCH_3	CH_3	H	162—163 (T)	56	$C_{16}H_{25}ClN_4O_2$	56.38 (56.33	7.39 7.56	16.44 16.46	10.40 10.36)	52
82	CH_3	NCH_3	CH_3	H	160—163 (AC)	61	$C_{15}H_{23}ClN_4O_2$	55.13 (54.83	7.09 7.25	17.14 16.90	10.85 11.18)	25
83	C_2H_5	NC_2H_5	C_2H_5	H	208—210 (M)	[c]	$C_{18}H_{29}ClN_4O_2 \cdot C_4H_4O_4$[f]	54.49 (54.34	6.86 6.83	11.55 11.30	7.31 7.46)	0
84	CH_3	NC_2H_5	C_2H_5	H	173—177 (E–DE)	[c]	$C_{17}H_{27}ClN_4O_2 \cdot 1.5C_4H_4O_4$[f] $\cdot 0.25H_2O$	51.78 (51.73	6.33 6.49	10.55 10.66	6.65 6.91)	20
85[i]	C_2H_5	O	$PhCH_2$	H								0
86[j]	C_2H_5	S	$PhCH_2$	H								2
87	CH_3	S	$PhCH_2$	H	137—140 (E)	46	$C_{20}H_{24}ClN_3O_2S \cdot 0.25H_2O$[j]	58.53 (58.46	6.02 5.93	10.24 10.29	8.64 8.71)	0
88	CH_3	SO	$PhCH_2$	H	205—210 (E)	[c]	$C_{20}H_{24}ClN_3O_3S \cdot 0.5H_2O$[k]	55.74 (55.64	5.85 5.61	9.75 9.64	8.23 8.20)	0
89[i]	C_2H_5	CH_2	$PhCH_2$	H								0
90[i]	C_2H_5	NCH_3	$PhCH_2$	H								48

a) Abbreviations for the solvents are as follows: I = isopropanol, M = methanol, W = water, E = ethanol, T = toluene, DE = diethyl ether, AC = acetone. *b*) Yields are given for the amine condensation and were not optimized. *c*) See Experimental. *d*) Oxalic acid. *e*) The relative stereochemistry is *trans*. *f*) Fumaric acid. *g*) The relative stereochemistry is *cis*. *h*) See ref. 44. *i*) See ref. 32. *j*) Calcd for S: 7.81, Found: 7.91. *k*) Calcd for S: 7.44, Found: 7.37.

bearing pyrrolidine, 4*H*-1,4-thiazine, piperidine, and piperazine rings in the amine part were prepared (**66**—**90**, Table 4). None of the benzamides prepared showed potent 5-HT_3 receptor antagonistic activity, and neither did the morpholinyl benzamides, whereas compounds **80**, **81**, and **90** with a piperazine ring showed weak activity. From these results, it is suggested that there is a slight interaction between the one nitrogen atom of the piperazine ring and the 5-HT_3 receptor.

Replacement of five- and six-membered heteroalicycles by a seven-membered ring, including hexahydro-1,4-oxazepine, -1,4-thiazine, or -1*H*-azepine (giving compounds **91**—**94**, Table 5), also provided no favorable effect. However, introduction of a hexahydro-1*H*-1,4-diazepine ring (**95**—**110**, Table 5) generally caused a remarkable increase in activity, although the reason for this is not clear. Interestingly, the 1,4-dimethyl- (**96**, **97**), 1,4-diethyl- (**101**, **102**), and 1-benzyl-4-methyl- (**103**, **104**) 1,4-diazepine derivatives showed much more potent 5-HT_3 receptor antagonistic activity than mosapride (ED_{50} = 261 μg/kg, i.v.) and metoclopramide (ED_{50} = 224 μg/kg, i.v.). On the other hand, the 1,4-dibenzyl-1,4-diazepine **95** showed very

Table 5. Physical Data and Serotonin-3 Receptor Antagonistic Activity for 2-Alkoxy-4-amino-5-chlorobenzamides (**91**—**110**)



Compd.	R	X	R_1	Y	R″	mp (°C) (Recryst. solvent[a])	Yield[b] (%)	Formula	Analysis (%) Calcd (Found) C	H	N	Cl	Inhibition of B–J reflex[c] (%) (μg/kg, i.v.) [ED_{50} (95% C.L.)]
91[d]	C_2H_5	O	$PhCH_2$	H_2	H								0 (100)
92[d]	C_2H_5	S	$PhCH_2$	H_2	H								3 (100)
93[d]	C_2H_5	CH_2	$PhCH_2$	H_2	H								0 (100)
94	C_2H_5	$CHCH_3$[e]	$PhCH_2$	H_2	H	169—171 (E)	63	$C_{23}H_{30}ClN_3O_2 \cdot C_4H_4O_4$[f]	60.95 (61.30	6.44 6.62	7.90 8.16	6.66 6.98)	0 (100)
95	C_2H_5	NCH_2Ph	$PhCH_2$	H_2	H	135—137 (E)	47	$C_{28}H_{33}ClN_4O_2 \cdot 0.5H_2O$	67.59 (67.64	6.79 6.71	11.26 11.30	7.13 7.11)	10 (100)
96	C_2H_5	NCH_3	CH_3	H_2	H	192—194 (E)	65	$C_{16}H_{25}ClN_4O_2 \cdot C_4H_4O_4$[f] $\cdot 0.5H_2O$	52.06 (51.98	6.44 6.47	12.14 11.92	7.68 7.56)	72 (1.0) [0.37 (0.12—1.11)]
97	CH_3	NCH_3	CH_3	H_2	H	184—186 (AC)	71	$C_{15}H_{23}ClN_4O_2 \cdot 0.25H_2O$	54.38 (54.45	7.15 7.10	16.91 16.84	10.70 10.85)	29 (1.0)
98	n-C_3H_7	NCH_3	CH_3	H_2	H	126—127 (T)	51	$C_{17}H_{27}ClN_4O_2$	57.54 (57.45	7.67 7.96	15.79 15.61	9.99 9.88)	5 (30)
99	iso-C_3H_7	NCH_3	CH_3	H_2	H	118—120 (E)	53	$C_{17}H_{27}ClN_4O_2$ $\cdot 2C_4H_4O_4$[f] $\cdot 0.7C_2H_5OH$[g]	51.20 (51.03	6.38 6.23	9.05 8.96	5.72 5.76)	90 (30)
100	CH_3	NCH_3	CH_3	H_2	CH_3	154—157 (E)	88	$C_{17}H_{27}ClN_4O_2$ $\cdot 2.25C_4H_4O_4$[f] $\cdot 0.25H_2O$	50.32 (50.35	5.93 5.66	9.03 8.84	5.71 6.00)	10 (30)
101	C_2H_5	NC_2H_5	C_2H_5	H_2	H	112—115 (E)	c)	$C_{18}H_{29}ClN_4O_2$ $\cdot 1.5C_4H_4O_4$[f] $\cdot 0.5H_2O$	52.22 (52.51	6.57 6.72	10.15 9.84	6.42 6.35)	42 (3.0) [4.5 (1.74—11.8)]
102	CH_3	NC_2H_5	C_2H_5	H_2	H	94—97 (I–DE)	c)	$C_{17}H_{27}ClN_4O_2$ $\cdot 2C_4H_4O_4$[f]	51.15 (51.44	6.01 6.20	9.54 9.53	6.04 6.19)	24 (3.0) [4.5 (1.80—11.2)]
103[d]	C_2H_5	NCH_3	$PhCH_2$	H_2	H								50 (1.0) [0.44 (0.11—1.77)]
104	CH_3	NCH_3	$PhCH_2$	H_2	H	99—104 (E–DE)	49	$C_{21}H_{27}ClN_4O_2$ $\cdot C_4H_4O_4$[f] $\cdot 0.5H_2O$	57.25 (57.23	6.22 6.43	10.47 10.22	6.63 6.69)	54 (1.0) [0.86 (0.14—5.20)]
105	C_2H_5	NCH_3	$PhCH_2$	O	H	165—167 (E–DE)	64	$C_{22}H_{27}ClN_4O_3 \cdot 0.25H_2O$	60.68 (60.90	6.37 6.22	12.87 12.74	8.14 8.27)	0 (100)
106	C_2H_5	NCH_3	2-$FC_6H_4CH_2$	H_2	H	70—73 (E–DE)	60	$C_{22}H_{28}ClFN_4O_2$ $\cdot C_2H_2O_4$[h] $\cdot 1.25H_2O$[i]	52.65 (52.95	5.98 6.11	10.23 10.31	6.48 6.22)	36 (1.0)
107	C_2H_5	NCH_3	3-$FC_6H_4CH_2$	H_2	H	78—82 (E–DE)	64	$C_{22}H_{28}ClFN_4O_2$ $\cdot 1.25C_2H_2O_4$[h] $\cdot 1.25H_2O$[j]	51.63 (51.66	5.84 5.62	9.83 9.77	6.22 6.46)	41 (1.0)
108	C_2H_5	NCH_3	4-$FC_6H_4CH_2$	H_2	H	90—93 (E–DE)	61	$C_{22}H_{28}ClFN_4O_2$ $\cdot C_2H_2O_4$[h] $\cdot 1.5H_2O$[k]	52.22 (51.94	6.17 5.82	9.94 10.05	6.29 6.51)	20 (1.0)
109	C_2H_5	NC_2H_5	$PhCH_2$	H_2	H	153—157 (E)	68	$C_{23}H_{31}ClN_4O_2$ $\cdot 2C_4H_4O_4$[f] $\cdot 0.5H_2O$	52.30 (52.13	5.85 5.87	9.04 8.77	5.72 5.68)	24 (30)
110	CH_3	NC_2H_5	$PhCH_2$	H_2	H	145—151 (E–DE)	67	$C_{22}H_{29}ClN_4O_2$ $\cdot 2C_4H_4O_4$[f] $\cdot 0.5H_2O$	51.53 (51.38	5.66 5.52	9.25 9.14	5.85 5.80)	9 (30)

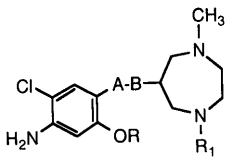
a) Abbreviations for the solvents are as follows: E=ethanol, I=isopropanol, T=toluene, DE=diethyl ether, AC=acetone. *b*) Yields are given for the amine condensation and were not optimized. *c*) See Experimental. *d*) See ref. 32. *e*) Diastereomeric mixture. *f*) Fumaric acid. *g*) The presence of ethanol was shown by the ^{1}H-NMR spectrum. *h*) Oxalic acid. *i*) Calcd for F: 3.47, Found: 3.09. *j*) Calcd for F: 3.33, Found: 3.13. *k*) Calcd for F: 3.37, Found: 2.99.

weak activity. This result suggested that there is a small lipophilic pocket in the receptor near the 4-position of the 1,4-diazepine ring. Furthermore, replacement of a nitrogen atom at the 4-position of **103** by a carbon atom (yielding **94**) caused a drastic decrease in activity, indicating that the 4-nitrogen atom of a seven-membered ring is essential for high activity. The presence of such a nitrogen atom might reflect the interaction with the receptor, such as hydrogen bonding. Among compounds described above, compounds **96** ($ED_{50}=0.37\,\mu g/kg$, i.v.) and **103** ($ED_{50}=0.44\,\mu g/kg$, i.v.) were substantially equipotent to tropisetron ($ED_{50}=0.39\,\mu g/kg$, i.v.) and superior to ondansetron ($ED_{50}=1.10\,\mu g/kg$, i.v.). The pharmacophore for 5-HT_3 receptor antagonists is regarded as an aromatic ring, a carbonyl function, and a basic nitrogen.[41] Thus the pharmacophore of tropisetron, ondansetron, **96**, and **103** is assumed to occupy the same relative position in space and probably interacts directly with the 5-HT_3 receptor.

A series of 2-ethoxybenzamides was somewhat more potent than the corresponding 2-methoxybenzamides (**96** *vs.* **97**, **101** *vs.* **102**, **103** *vs.* **104**). In a 1,4-diazepinyl benzamide series, introduction of a methyl group into the 6-position of the 1,4-diazepine ring (giving **100**) and oxidation of compound **103** (yielding **105**) led to a profound decrease compared with each parent compound. This result may be attributable to an unfavorable conformation of these compounds in relation to the 5-HT_3 receptor. Although the 2-propoxybenzamides **98** and **99** having a 1,4-dimethyl-1,4-diazepine ring and introduction of a fluoro atom into the benzyl group of **103** (giving **106**—**108**) and replacement of a methyl group in the 1,4-diazepine ring of **103** and **104** by an ethyl group (giving **109** and **110**, respectively) resulted in a slight decrease of the activity, compounds **106**—**108** still retained high activity.

Next, in order to examine the importance of the amide moiety of **96** and **103**, the corresponding esters **111**—**114**

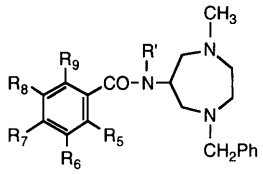
Table 6. Physical Data and Serotonin-3 Receptor Antagonistic Activity for Compounds **111**—**115**



Compd.	R	R_1	A–B	mp (°C) (Recryst. solvent)	Yield[a] (%)	Formula	Analysis (%) Calcd (Found) C	H	N	Cl	Inhibition of B–J reflex[b] (%) (μg/kg, i.v.)
111	C_2H_5	CH_3	CO–O	152—155 (iso-PrOH)	67	$C_{16}H_{24}ClN_3O_3 \cdot 0.75(CH_3)_2CHOH^{c)} \cdot 0.75H_2O$	49.84 (50.09	6.29 6.49	6.64 6.43	5.60 5.23)	2 (100)
112	CH_3	CH_3	CO–O	162—164 (EtOH)	45	$C_{15}H_{22}ClN_3O_3 \cdot 2.5C_4H_4O_4^{d)} \cdot 0.5H_2O$	47.89 (47.76	5.31 5.60	6.70 6.71	5.65 5.55)	1 (100)
113	C_2H_5	$PhCH_2$	CO–O	155—157 (MeOH–Et_2O)	26	$C_{22}H_{28}ClN_3O_3$	52.86 (52.76	6.25 6.40	8.41 8.26	21.28 21.00)	40 (1.0)
114	CH_3	$PhCH_2$	CO–O	149—152 (MeOH–Et_2O)	23	$C_{21}H_{26}ClN_3O_3 \cdot 2HCl \cdot 0.5H_2O$	51.44 (51.49	6.06 6.18	8.57 8.49	21.69 21.56)	0 (100)
115	CH_3	$PhCH_2$	NH–CO	126—128 (EtOH)	[b]	$C_{21}H_{27}ClN_4O_2$	62.60 (62.63	6.75 6.64	13.91 13.89	8.80 8.73)	17 (100)

a) Yields are given for the alcohol condensation and were not optimized. *b*) See Experimental. *c*) The presence of isopropanol was shown by the ^{1}H-NMR spectrum. *d*) Fumaric acid.

Table 7. Physical Data and Serotonin-3 Receptor Antagonistic Activity for Benzamides **116**—**129**



Compd.	R′	R_5	R_6	R_7	R_8	R_9	mp (°C) (Recryst. solvent[a])	Yield[b] (%)	Formula	Analysis (%) Calcd (Found) C	H	N	Cl	Inhibition of B–J reflex[c] (%) (mg/kg, i.v.) [ED_{50} (95% C.L.)]
116	H	$OCH_2CH_2CH_3$	H	NH_2	Cl	H	90—91 (E–DE)	62	$C_{23}H_{31}ClN_4O_2 \cdot 2C_4H_4O_4^{d)} \cdot 0.5H_2O$	52.68 (52.62	5.81 5.84	9.10 8.98	5.76 5.72)	0 (100)
117	H	$OCH(CH_3)_2$	H	NH_2	Cl	H	100—103 (M–DE)	55	$C_{23}H_{31}ClN_4O_2 \cdot 2C_4H_4O_4^{d)}$	53.07 (52.81	5.77 5.95	9.17 9.19	5.80 5.90)	0 (100)
118	H	OC_2H_5	H	$NHCOCH_3$	Cl	H	87—90 (E–DE)	43	$C_{24}H_{31}ClN_4O_3 \cdot C_2H_2O_4^{e)} \cdot 1.5H_2O$	54.21 (54.26	6.30 6.05	9.73 9.46	6.15 6.08)	7 (100)
119		CO–O	H	NH_2	Cl	H	205—208 (E)	[c]	$C_{21}H_{23}ClN_4O_3 \cdot 0.25H_2O$	60.07 (60.31	5.65 5.52	13.36 13.32	8.45 8.58)	48 (100)
120	H	OCH_3	OCH_3	H	H	H	77—81 (E)	51	$C_{22}H_{29}N_3O_3 \cdot 2C_2H_2O_4^{e)} \cdot H_2O$	53.70 (53.80	6.07 6.03	7.23 6.83)		0 (10)
121	H	OCH_3	H	H	Br	OH	79—83 (EA)	22	$C_{21}H_{26}BrN_3O_3 \cdot 0.25H_2O^{f)}$	55.70 (52.81	5.90 5.95	9.28 9.19)		0 (10)
122	H	H	Cl	H	Cl	H	149—151 (M)	60	$C_{20}H_{23}Cl_2N_3O \cdot 2C_2H_2O_4^{e)}$	50.36 (50.14	4.75 4.81	7.34 7.15	12.39 12.00)	11 (10)
123	H	OCH_3	Cl	H	Cl	OCH_3	80—83 (DE)	55	$C_{22}H_{27}Cl_2N_3O_3$	58.41 (58.33	6.02 5.96	9.29 9.27	15.67 15.56)	4 (10)
124	H	OCH_3	Br	H	H	OCH_3	106—109 (E)	60	$C_{22}H_{28}BrN_3O_3 \cdot 2C_2H_2O_4^{e)} \cdot 0.75H_2O^{g)}$	47.61 (47.66	5.15 5.27	6.41 6.15)		23 (10)
125	H	OCH_3	H	H	Cl	OCH_3	102—104 (E)	55	$C_{22}H_{28}N_3O_3 \cdot 1.75C_2H_2O_4^{e)} \cdot 1.5H_2O$	50.83 (51.00	5.77 5.59	6.97 6.61	5.88 6.23)	18 (10)
126	H	OCH_3	H	H	H	OCH_3	85—89 (E)	16	$C_{22}H_{29}N_3O_3 \cdot 2C_2H_2O_4^{e)} \cdot 0.75H_2O$	54.12 (54.49	6.03 6.20	7.28 6.93)		0 (10)
127	H	OCH_2–CH_2		H	H	H	72—76 (E–DE)	68	$C_{22}H_{27}N_3O_2 \cdot 1.5C_2H_2O_4^{e)} \cdot 0.25H_2O$	59.46 (59.51	6.09 6.10	8.32 8.42)		17 (100)
128	H	OCH_2–CH_2		H	Br	H	110 (AC)	23	$C_{22}H_{26}BrN_3O_2^{h)}$	59.46 (59.48	5.90 5.87	9.46 9.40)		95 (100) [10.3 (4.90—21.8)]
129	H	OCH_2–CH_2		H	NO_2	H	142—143 (CH–AC)	40	$C_{22}H_{26}N_4O_4$	64.37 (64.10	6.38 6.33	13.65 13.53)		68 (100)

a) Abbreviations for the solvents are as follows: E=ethanol, DE=diethyl ether, M=methanol, EA=ethyl acetate, AC=acetone, CH=chloroform. *b*) Yields are given for the amine condensation and were not optimized. *c*) See Experimental. *d*) Fumaric acid. *e*) Oxalic acid. *f*) Calcd for Br: 17.65, Found: 17.40. *g*) Calcd for Br: 12.18, Found: 12.02. *h*) Calcd for Br: 17.98, Found: 17.89.

Table 8. Protection against Cisplatin-Induced Emesis in Ferrets

Compd.	mg/kg, i.v. ×2[a]	Protection[b]	Latency to emetic episodes (min) Mean±S.E.	Number of emetic episodes Mean±S.E.
Saline		0/6	57.5± 3.9	13.2±1.7
96	0.1	0/4	156.3±14.4[d]	1.8±1.2[d]
103	0.1	2/4	114.3± 5.5[d]	5.5±0.6[c]
Ondansetron	0.03	3/6	148.0±14.6[d]	1.2±0.7[d]
	0.1	4/4	>180[d]	0[d]
Granisetron	0.03	0/4	101.0± 9.5[c]	5.3±1.5[c]
	0.1	5/5	>180[d]	0[d]

a) Treatment schedule: first dose 30 min before, followed by second dose, 45 min after cisplatin. *b*) Number of ferrets completely protected/ferrets used. The superscripts *c* and *d* indicate a statistically significant difference from the saline control (Williams–Wilcoxon's multiple test). *c*) $p<0.05$. *d*) $p<0.01$.

Table 9. Serotonin Receptor Binding Assay[a]

Compd.	5-HT_3 binding affinity IC_{50} (nM)	5-HT_4 binding affinity IC_{50} (nM)
96	3.4	>1000
103	0.88	>1000
Ondansetron	4.2	>1000
Granisetron	2.0	>1000
Mosapride	1380	113

a) Experiments were performed as described in Experimental.

and acetamide **115**, corresponding to a reversal of the amide linkage, were prepared (Table 6). All these compounds were less potent than the parent compounds. However, 1,4-dimethylhexahydro-1*H*-1,4-diazepin-6-yl 4-amino-5-chloro-2-ethoxybenzoate (**113**) showed potent activity. This result suggests that the active conformation of **111**, **112**, **114**, and **115** is not similar to those of **96** and **103**. The influence of substituents of the benzoyl group of **103** on the B–J reflex was finally studied, while keeping the 1-benzyl-4-methylhexahydro-1*H*-1,4-diazepine ring constant (Table 7). Replacement of an ethoxy group at the 2-position by a propoxy group (**116**, **117**) and of an amino group at the **4**-position by an acetylamino group (**118**) resulted in weaker activity. Formation of benzoxazine (**119**) and benzofuran (**127**—**129**) rings resulted in moderate activity. On the other hand, many combinations of methoxy and halogeno groups (**120**—**126**) gave decreased activity. Overall, it was found that a 2-methoxy- or 2-ethoxy-4-amino-5-chlorobenzamide moiety was essential for potent 5-HT_3 receptor antagonistic activity as well as potent 5-HT_4 receptor agonist activity, as in the case of mosapride.[29,42]

On the basis of the B–J reflex activity, compounds **96** and **103** were selected for further biological assay involving protection against cisplatin-induced emesis in ferrets and activities of 5-HT_3 receptor antagonism and 5-HT_4 receptor agonism *in vitro*. The results are shown in Tables 8 and 9. In Table 8, the activities of granisetron and ondansetron are included for comparison. Compounds **96** and **103** did not completely inhibit the emetic episodes induced by cisplatin at 0.1 mg/kg, i.v. On the other hand, granisetron and ondansetron completely inhibited the emetic episodes induced by cisplatin at the same dose. Overall, compounds **96** and **103** were somewhat less potent than ondansetron and granisetron, but were much more potent than mosapride and metaclopromide (see Table 2). Furthermore, from the [^{3}H]quipazine and [^{3}H]GR113808 binding tests (Table 9), it was found that compounds **96** and **103** showed potent 5-HT_3 receptor binding affinity without 5-HT_4 receptor binding affinity, like granisetron and ondansetron.

In summary, modification of the amine moiety of mosapride led to many compounds with better 5-HT_3 receptor activity than metoclopramide. Among them, 4-amino-5-chloro-*N*-(1,4-dimethylhexahydro-1*H*-1,4-diazepin-6-yl)-2-ethoxybenzamide (**96**) and the 1-benzyl-4-methylhexahydro-1*H*-1,4-diazepine analogue **103** were found to possess potent 5-HT_3 receptor antagonistic activity without 5-HT_4 receptor activity. The synthesis and biological activities of further series of 1,4-diazepinyl derivatives will be reported in due course.

Experimental

Chemistry All melting points were determined on a Yanagimoto micromelting point apparatus without correction. IR spectra were recorded on a Hitachi 260-10 spectrometer. Electron ionization and secondary ion mass spectra were obtained on a JEOL JMS D-300 or a Hitachi M-80-B spectrometer. ^{1}H-NMR spectra were taken at 80 MHz with a Varian FT-80A spectrometer, at 200 MHz with a Varian Gemini-200 spectrometer, and at 300 MHz with a Varian XL-300 spectrometer. Chemical shifts are expressed as δ (ppm) values with $SiMe_4$ as an internal standard, and coupling constants (J) are given in Hz. Organic extracts were dried over anhydrous $MgSO_4$ or anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure. Merck Silica gel 60 (70—230 mesh) was used for column chromatography, and Yamazen YFLC gel Y-1-1064 (40—63 μm) was used for medium-pressure silica gel column chromatography. The following known amine, alcohol, and benzoic acid derivatives were prepared according to the literature: 3-(aminomethyl)-1-benzylpyrrolidine (**9a**),[43] *trans*-3-(aminomethyl)-1-benzyl-4-methylpyrrolidine (**9b**),[43] *cis*-3-(aminomethyl)-1-benzyl-4-methylpyrrolidine (**9c**),[43] *trans*-3-(aminomethyl)-1-benzyl-4-ethylpyrrolidine (**9d**),[43] 2-(aminomethyl)-4-benzyltetrahydro-4*H*-1,4-thiazine (**10a**),[44] 3-(aminomethyl)-1-benzylpiperidine (**10b**),[44] 3-[(acetylamino)methyl]-1-benzyltetrahydropyridin-3-ene (**10c**),[44] 3-(aminomethyl)-4-benzyltetrahydro-4*H*-1,4-thiazine (**11**),[32] 6-amino-1-benzyl-4-methylhexahydro-1*H*-1,4-diazepine (**24a**),[32] 6-amino-1,4-dimethylhexahydro-1*H*-1,4-diazepine (**24b**),[31] 6-amino-1-benzyl-4-methyl-7-oxohexahydro-1*H*-1,4-diazepine (**30**),[39] 1,4-dimethylhexahydro-1*H*-1,4-diazepin-6-ol (**33**),[31] 4-amino-5-chloro-2-ethoxybenzoic acid (**40b**),[28a] 5-bromo-6-hydroxy-2-methoxybenzoic acid (**40e**),[45] 3,5-dichloro-2,6-dimethoxybenzoic acid (**40g**),[46] 3-bromo-2,6-dimethoxybenzoic acid (**40h**),[46] 5-chloro-2,6-dimethoxybenzoic acid (**40i**),[46] 2,3-dihydrobenzofuran-7-carboxylic acid (**40j**),[47] 5-chloro-2,3-dihydrobenzofuran-7-carboxylic acid (**40k**),[47] 5-nitro-2,3-dihydrobenzofuran-7-carboxylic acid (**40l**).[47] 4-Amino-5-chloro-2-methoxybenzoic acid (**40a**), 2,3-dimethoxybenzoic acid (**40c**), 3,5-dichlorobenzoic acid (**40d**), and 2,6-dimethoxybenzoic acid (**40f**) were obtained from commercial suppliers.

1-[(2-Chlorobenzyl)amino]-2-propanol (2a) A mixture of 1-amino-2-propanol (**1a**, 25.0 g, 0.33 mol), 2-chlorobenzaldehyde (51.5 g, 0.37 mol), $NaHCO_3$ (33.6 g, 0.40 mol), and MeOH (1000 ml) was heated to reflux for 4 h and then cooled to *ca.* 10 °C. Sodium borohydride (13.9 g, 0.37 mol) was added portionwise to the stirred reaction mixture during a period of 2 h at *ca.* 10 °C. The whole was stirred at the same temperature for 0.5 h and at room temperature for 1 h. The insoluble materials were filtered off, and then the filtrate was concentrated to dryness. The residue was dissolved in $CHCl_3$ and the solution was washed successively with water and brine. The solvent was evaporated to give 65.0 g (98%) of **2a** as an oil, which was used in the next step without further purification. ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 1.15 (d, J=6.5, 3H, CH_3), 2.25 (br s, 1H), 2.42 (dd, J=9.5, 12.5, 1H, 1-C$\underline{H}_2$), 2.72 (ddd, J=0.5, 3.0, 12.5, 1H, 1-C$\underline{H}_2$), 3.8 (m, 1H, 2-CH), 3.90 (s, 2H, C$\underline{H}_2C_6H_4Cl$), 7.1—7.3, 7.3—7.45 (m, 4H, arom H). IR (neat) ν cm^{-1}: 3300, 2950, 2900, 1430. MS *m/z*: 200 (MH^+).

2-[(2-Chlorobenzyl)amino]-2-methyl-1-propanol (2c) In a similar

manner to that described above, compound **2c** was prepared from 2-amino-2-methyl-1-propanol (**1c**) and 2-chlorobenzaldehyde. ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 1.16 (s, 6H, $CH_3 \times 2$), 1.9 (br s, 1H), 3.38 (s, 2H, 1-CH_2), 3.77 (s, 2H, $C\underline{H}_2C_6H_4Cl$), 7.15—7.3, 7.3—7.45 (m, 4H, arom H). IR (neat) νcm^{-1}: 3350, 2970, 2870, 1440. MS *m/z*: 214 (MH^+).

2-(Aminomethyl)-4-(2-chlorobenzyl)-6-methylmorpholine (4a) A mixture of **2a** (5.8 g, 29 mmol) and *N*-(2,3-epoxypropyl)phthalimide (**3**, 6.2 g, 31 mmol) was heated at 80 °C for 3 h. Concentrated H_2SO_4 (15.7 g, 0.16 mol) was gradually added to the resultant oil, and the mixture was rapidly heated at *ca.* 150 °C and kept at the same temperature for 2 h. The resulting reaction mixture was cooled at room temperature, and then ice-water was added. The insoluble materials were filtered off, and the filtrate was basified with 48% aqueous NaOH and extracted with $CHCl_3$. The extract was washed successively with water and brine, and concentrated to give 3.6 g (49%) of **4a** as an oil. A part of this oil was acetylated with Ac_2O to give 2-[(acetylamino)methyl]-4-(2-chlorobenzyl)-6-methylmorpholine as an oil. ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 1.14 (d, J=6.5, 3H, CH_3), 1.60—2.30 (m, 3H), 2.00 (s, 3H, $COCH_3$), 2.70—2.85 (m, 2H), 3.09 (m, 1H), 3.40—3.85 (m, 2H), 3.62 (s, 2H, $C\underline{H}_2C_6H_4Cl$), 5.87 (br s, 1H, CONH), 7.10—7.30 (m, 2H, arom H), 7.35 (m, 1H, arom H), 7.42 (m, 1H, arom H). IR (neat) νcm^{-1}: 1640. MS *m/z*: 297 (MH^+).

In a similar manner to that described above, compounds **4b—e** were prepared from 2-(benzylamino)-1-propanol (**2b**),[48] **2c**, 2-(benzylamino)-2-phenylethanol (**2d**),[49] and 3-(benzylamino)-1-propanol (**2e**),[50] respectively.

1-[(*N*-Benzyl-*N*-chloroacetyl)amino]-3-phthalimido-2-propanol (6) A mixture of **3** (10.0 g, 49 mmol) and benzylamine (15.8 g, 0.15 mol) was stirred at room temperature for 0.5 h. The oil containing 1-(benzylamino)-3-phthalimido-2-propanol (**5**) was dissolved in CH_2Cl_2 (300 ml) and, Et_3N (22.5 g, 0.22 mol) was added. Chloroacetyl chloride (16.7 g, 0.15 mol) was added dropwise to the cold solution (−5 °C). The mixture was stirred at the same temperature for 1 h and then at room temperature for 2 h. The solution was washed successively with water, 10% aqueous NaOH, 10% HCl, water, and brine and concentrated to dryness. The resultant oil was chromatographed on silica gel with $CHCl_3$ to afford 10.0 g (53%) of **6** as an oil. ^{1}H-NMR (80 MHz, $CDCl_3$) δ: 3.0—3.75 (m, 5H), 4.00 (s, 2H, $COCH_2Cl$), 4.32 (d, J=11, 1H, $C\underline{H}_2Ph$), 4.52 (d, J=11, 1H, $C\underline{H}_2Ph$), 6.90—7.90 (m, 9H, arom H). IR (neat) νcm^{-1}: 1665 ($COCH_2$). MS *m/z*: 386 (M^+).

***N*-[(4-Benzyl-5-oxo-2-morpholinyl)methyl]phthalimide (7)** Sodium hydride (60% dispersion in mineral oil, 1.2 g, 30 mmol) was added portionwise to a solution of **6** (10.0 g, 26 mmol) in anhydrous tetrahydrofuran (150 ml) at −10 °C. The mixture was stirred at the same temperature for 0.5 h and then at room temperature for 2 h. The solvent was evaporated, and the residue was dissolved in $CHCl_3$. The solution was washed successively with water and brine, and concentrated to dryness. The residue was chromatographed on silica gel with $CHCl_3$: MeOH = 30 : 1 to give 5.4 g (60%) of **7** as an oil. ^{1}H-NMR (80 MHz, $CDCl_3$) δ: 2.75—3.40 (m, 4H), 3.55 (m, 1H), 4.06 (d, J=7, 1H, $C\underline{H}_2Ph$), 4.40 (s, 2H, 6-CH_2), 4.45 (d, J=7, 1H, $C\underline{H}_2Ph$), 7.0—7.8 (m, 9H, arom H). IR (neat) νcm^{-1}: 1670 (CON). MS *m/z*: 350 (M^+).

2-(Aminomethyl)-4-benzyl-5-oxomorpholine (8) A mixture of **7** (4.0 g, 11 mmol), 85% $NH_2NH_2 \cdot H_2O$ (850 mg, 14 mmol), and EtOH (40 ml) was heated to reflux for 2 h and then cooled to room temperature. The reaction mixture was diluted with $CHCl_3$, and the precipitates were filtered off. The filtrate was washed successively with small amounts of water and brine. The solvent was evaporated to give quantitatively 2.5 g of **8** as an oil, which was used in the next step without further purification. MS *m/z*: 221 (MH^+).

2-(Aminomethyl)-1,4-dimethylpiperazine (13) A mixture of *N*-[(1,4-dimethyl-2-piperazinyl)methyl]phthalimide[31] (**12**, 6.0 g, 22 mmol), 100% $NH_2NH_2 \cdot H_2O$ (1.1 g, 22 mol), and EtOH (90 ml) was heated to reflux for 2.5 h and then cooled to room temperature. The reaction mixture was diluted with $CHCl_3$, and the precipitates were filtered off. The filtrate was washed successively with small amounts of water and brine. The solvent was evaporated to give quantitatively 3.0 g of **13** as an oil, which was used in the next step without further purification. MS *m/z*: 144 (MH^+).

2-[(Acetylamino)methyl]-1,4-dibenzylpiperazine (15a) and 6-(Acetylamino)-1,4-dibenzylhexahydro-1*H*-1,4-diazepine (16a) A mixture of 2-(chloromethyl)-1,4-dibenzylpiperazine[33] (**14a**, 7.5 g, 24 mmol), NaN_3 (3.1 g, 48 mmol), and CH_3CN (75 ml) was heated to reflux for 4 h and then cooled to room temperature. The insoluble materials were filtered off, and the filtrate was concentrated to dryness. The residue was dissolved in toluene (70 ml), and sodium bis(2-methoxyethoxy)aluminum hydride (Vitride®, 70% solution in toluene; 6.8 g, 22 mmol) was added dropwise at 5 °C. The mixture was stirred at the same temperature for 2 h. The excess of the reducing agent was decomposed by addition of 20% aqueous NaOH. The organic layer was separated, then washed successively with water and brine. To the dry solution was added Ac_2O (4.4 g, 43 mmol). The mixture was stirred at room temperature for 3 h and then washed successively with 10% aqueous NaOH, water, and brine. The solvent was evaporated to give a mixture of **15a** and **16a** as an oil. The mixture was separated by medium-pressure silica gel column chromatography ($CHCl_3$: MeOH = 30 : 1) to give 1.3 g (16%) of **16a** and 3.3 g (41%) of **15a** in that order.

Compound **15a** (Oil): ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 1.94 (s, 3H, $COCH_3$), 2.25—2.90 (m, 7H), 3.30—3.60 (m, 2H), 3.40 (d, J=14.0, 1H, $C\underline{H}_2Ph$), 3.50 (s, 2H, CH_2Ph), 3.95 (d, J=14.0, 1H, $C\underline{H}_2Ph$), 6.32 (br s, 1H, CONH), 7.22—7.41 (m, 10H, arom H). IR (KBr) νcm^{-1}: 1640. MS *m/z*: 338 (MH^+), 265 ($M^+ - CH_2NHCOCH_3$).

Compound **16a**: mp 65—68 °C (toluene). ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 1.78 (s, 3H, $COCH_3$), 2.48—2.83 (m, 6H), 2.90 (dd, J=13.0, 4.0, 2H), 3.53 (d, J=13.0, 2H, $C\underline{H}_2Ph \times 2$), 3.69 (d, J=13.0, 2H, $C\underline{H}_2Ph \times 2$), 3.96 (m, 1H), 6.25 (d, J=8.0, 1H, NHCO), 7.32 (m, 10H, arom H). IR (KBr) νcm^{-1}: 1640. *Anal.* Calcd for $C_{21}H_{27}N_3O$: C, 74.74; H, 8.06; N, 12.45. Found: C, 74.50; H, 7.85; N, 12.30. MS *m/z*: 338 (MH^+), 279 ($M^+ - NHCOCH_3$).

2-[(Acetylamino)methyl]-1,4-diethylpiperazine (15b) and 6-(Acetylamino)-1,4-diethylhexahydro-1*H*-1,4-diazepine (16b) In a similar manner to that described above, a mixture of **15b** and **16b** was prepared from 2-(chloromethyl)-1,4-diethylpiperazine[34] (**14b**) and used in the next step without further purification. The ratio (**15b** : **16b** = 8.7 : 1) was determined form the ^{1}H-NMR spectrum [300 MHz, $CDCl_3$; 6.29 (br s, 1H, $CH_2N\underline{H}COCH_3$ of **15b**), 6.80 (br s, 1H, $N\underline{H}COCH_3$ of **16b**)]. MS *m/z*: 214 (MH^+), 155 ($M^+ - NHCOCH_3$), 141 ($M^+ - CH_2NHCOCH_3$).

3-(Acetylamino)-1-benzyl-5-methylhexahydro-1*H*-azepine (20) A mixture of **17**[35] (6.0 g, 47 mmol), benzyl chloride (5.9 g, 47 mmol), anhydrous K_2CO_3 (13.0 g, 94 mmol), and methyl ethyl ketone (200 ml) was heated to reflux for 6 h and then cooled to room temperature. The insoluble materials were filtered off, and the filtrate was concentrated to dryness. The residue was chromatographed on silica gel with AcOEt : MeOH = 9 : 1 to give 6.5 g (64%) of 1-benzyl-4-methyl-2-piperidinemethanol (**18**) as an oil [MS *m/z*: 220 (MH^+)].

A mixture of **18** (3.2 g, 15 mmol), thionyl chloride (3.2 ml, 44 mmol), *N*,*N*-dimethylformamide (DMF, 0.1 ml), and CH_2Cl_2 (60 ml) was heated to reflux for 2 h and then cooled to room temperature. The reaction mixture was concentrated to dryness. The residue was dissolved in $CHCl_3$ and washed successively with saturated aqueous $NaHCO_3$, water, and brine. The solvent was evaporated to afford 3.4 g (98%) of 1-benzyl-2-(chloromethyl)-4-methylpiperidine (**19**) as an oil [MS *m/z*: 238 (MH^+)].

A mixture of **19** (3.4 g, 14 mmol), NaN_3 (1.9 g, 29 mmol), and CH_3CN (60 ml) was heated to reflux for 2 h and cooled to room temperature. The insoluble materials were filtered off, and the filtrate was concentrated to dryness [IR (neat) νcm^{-1}: 2090 (N_3)]. The residue was dissolved in toluene (50 ml), and then Vitride® (70% solution in toluene; 8.4 g, 29 mmol) was added dropwise at 5 °C. The mixture was stirred at the same temperature for 2 h. The excess of the reducing agent was decomposed by addition of 20% aqueous NaOH. The organic layer was separated, and washed successively with water and brine. To the dry solution was added Ac_2O (2.9 g, 28 mmol), and the mixture was stirred at room temperature for 3 h. The solution was washed successively with 10% aqueous NaOH, water, and brine. The solvent was evaporated to give a mixture of **20** and 2-[(acetylamino)methyl]-1-benzyl-4-methylpiperidine (**21**) as an oil. The mixture was separated by medium-pressure silica gel column chromatography ($CHCl_3$: MeOH = 100 : 1) to give 3.0 g (81%) of **20** and 0.6 g (16%) of **21**.

Compound **20** (Oil): ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.94 (d, J=6.0, 3H, CH_3), 1.25—1.80 (m, 3H), 1.72 (s, 3H, $COCH_3$), 1.95 (m, 1H), 2.44 (m, 1H), 2.64—2.80 (m, 2H), 3.00 (m, 1H), 3.52 (d, J=13.0, 1H, $C\underline{H}_2Ph$), 3.77 (d, J=13.0, 1H, $C\underline{H}_2Ph$), 3.98 (m, 1H), 5.90 (m, 1H), 7.24—7.39 (m, 5H, arom H). IR (neat) νcm^{-1}: 1645. MS *m/z*: 261 (MH^+), 202 ($M^+ - NHCOCH_3$).

Compound **21** (Oil): ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.94 (d, J=6.0, 3H, CH_3), 1.15—1.70 (m, 5H), 2.02 (s, 3H, $COCH_3$), 2.18 (m, 1H), 2.64 (m, 1H), 2.96 (d, J=13.0, 1H, $C\underline{H}_2Ph$), 3.32 (m, 1H), 3.40—3.58 (m, 2H), 4.06 (d, J=13.0, 1H, $C\underline{H}_2Ph$), 7.24—7.43 (m, 5H, arom H). IR

(neat) ν cm^{-1}: 1640. MS *m/z*: 261 (MH^+), 188 ($M^+ - CH_2NHCOCH_3$).

6-Amino-1,4,6-trimethylhexahydro-1*H*-1,4-diazepine (23) A solution of **22**[36] (9.3 g, 50 mmol) in 10% aqueous EtOH (200 ml) was hydrogenated over Raney Ni (wet, 3 g) at room temperature at 4.0 kg/cm^2, until no more hydrogen was consumed. The catalyst was filtered off, and the filtrate was evaporated to give 5.4 g (69%) of **23** as a pale brown oil. ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 0.99 (s, 3H, 6-CH_3), 1.60 (br s, 2H, NH_2), 2.31 (d, J=13.0, 2H, 5-C$\underline{H}_2$ and 7-C$\underline{H}_2$), 2.35 (s, 6H, N-CH_3 × 2), 2.48 (d, J=13.0, 2H, 5-C$\underline{H}_2$ and 7-C$\underline{H}_2$), 2.42—2.53 (m, 2H), 2.57—2.70 (m, 2H).

1-Benzyl-4-ethyl-6-nitrohexahydro-1*H*-1,4-diazepine (28) *N*-Benzyl-*N'*-ethylethylenediamine[34] (**26**, 78.6 g, 0.44 mol) was added dropwise to a mixture of tris(hydroxymethyl)nitromethane (**25**, 70.0 g, 0.46 mol), $NaHCO_3$ (23.4 g, 0.28 mol), and water (470 ml) at room temperature. The reaction mixture was heated at *ca.* 50 °C for 2 h, then cooled to room temperature, and extracted with CH_2Cl_2. The extract was washed successively with water and brine. The solvent was evaporated at *ca.* 35 °C to give 1-benzyl-4-ethyl-6-(hydroxymethyl)-6-nitrohexahydro-1*H*-1,4-diazepine (**27**) as a pale brown oil. This product was dissolved in MeOH (260 ml), and *tert*-BuOK (54.5 g, 0.49 mol) was added portionwise at 20 °C. The mixture was stirred at room temperature for 0.5 h and concentrated to dryness. A solution of 95% $NH_2OH \cdot HCl$ (33.8 g, 0.49 mol) in H_2O (200 ml) was added to the oily residue, and the mixture was immediately extracted with CH_2Cl_2 and washed with brine. The solvent was evaporated at *ca.* 30 °C to give a crude product, which was chromatographed on silica gel with ethyl acetate to afford 66.7 g (57% yield from **26**) of **28** as a pale brown oil. ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 1.60 (t, J=7.2, 3H, $NCH_2C\underline{H}_3$), 2.65 (q, J=7.2, 2H, $NC\underline{H}_2CH_3$), 2.50—2.82 (m, 4H), 3.20 (dd, J=5.5, 14.0, 1H, 5- or 7-C$\underline{H}_2$), 3.21 (dd, J=5.5, 14.0, 1H, 5- or 7-C$\underline{H}_2$), 3.38 (dd, J=1.5, 14.0, 1H, 5- or 7-C$\underline{H}_2$), 3.41 (dd, J=1.0, 14.0, 1H, 5- or 7-C$\underline{H}_2$), 3.69 (d, J=13.5, 1H, C$\underline{H}_2$Ph), 3.77 (d, J=13.5, 1H, C$\underline{H}_2$Ph), 4.49 (quint, J=6.0, 1H, 6-H), 7.20—7.35 (m, 5H, arom H).

6-(Acetylamino)-1-benzyl-4-ethylhexahydro-1*H*-1,4-diazepine (29) A solution of **28** (66.4 g, 0.25 mol) in EtOH (400 ml) was hydrogenated over Raney Ni (wet, 8 g) at room temperature and atmospheric pressure, until no more hydrogen was consumed. The catalyst was filtered off, and the filtrate was concentrated to dryness to leave an oily residue containing crude 6-amino-1-benzyl-4-ethylhexahydro-1*H*-1,4-diazepine. The residue was dissolved in $CHCl_3$ (200 ml) and then Ac_2O (13.7 g, 0.13 mol) was added. The mixture was stirred at room temperature for 3 h and washed successively with 10% aqueous NaOH, water, and brine. The solvent was evaporated to give the crude product, which was chromatographed on silica gel with $CHCl_3$: MeOH = 9 : 1 to afford 43.5 g (63%) of **29**. ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 1.05 (t, J=7.2, 3H, $NCH_2C\underline{H}_3$), 1.88 (s, 3H, $COCH_3$), 2.65 (q, J=7.2, 2H, $NC\underline{H}_2CH_3$), 2.40—3.00 (m, 8H), 3.56 (d, J=13.5, 1H, C$\underline{H}_2$Ph), 3.70 (d, J=13.5, 1H, C$\underline{H}_2$Ph), 4.03 (m, 1H, 6-CH), 6.49 (br s, 1H, NHCO), 7.20—7.40 (m, 5H, arom H). MS *m/z*: 276 (MH^+).

6-(Acetylamino)-1-(2-fluorobenzyl)-4-methylhexahydro-1*H*-1,4-diazepine (32a) A mixture of **24a** (2.7 g, 10 mmol), EtOH (50 ml), and CH_3COOH (AcOH, 5 ml) was hydrogenated over 10% palladium on carbon (0.3 g) at 50 °C. After the theoretical amount of hydrogen was absorbed, the catalyst was removed by filtration. The filtrate was concentrated to dryness, giving 6-(acetylamino)-1-methylhexahydro-1*H*-1,4-diazepine (**31**) as an oil. A mixture of **31** (*ca.* 1.7 g), 2-fluorobenzyl chloride (1.5 g, 10 mmol), anhydrous K_2CO_3 (12.0 g, 87 mmol), KI (0.2 g), and methyl ethyl ketone (200 ml) was heated to reflux for 16 h and then cooled to room temperature. The insoluble materials were filtered off, and the filtrate was concentrated to dryness. The residue was dissolved in $CHCl_3$ and washed successively with water and brine. The solvent was evaporated to leave an oil, which was chromatographed on silica gel with $CHCl_3$: MeOH = 20 : 1 to give 1.9 g (66% yield from **24a**) of **32a** as an oil. ^{1}H-NMR (80 MHz, $CDCl_3$) δ: 1.89 (s, 3H, NCH_3), 2.35 (s, 3H, $COCH_3$), 2.40—3.01 (m, 8H), 3.66 (s, 2H, CH_2Ph), 4.00 (m, 1H, 6-CH), 6.61 (m, 1H, NHCO), 6.85—7.45 (m, 4H, arom H). MS *m/z*: 279 (M^+).

6-(Acetylamino)-1-(3-fluorobenzyl)-4-methylhexahydro-1*H*-1,4-diazepine (32b) In a similar manner to that described above, compound **32b** was prepared from **31** and 3-fluorobenzyl chloride. ^{1}H-NMR (80 MHz, $CDCl_3$) δ: 1.90 (s, 3H, NCH_3), 2.36 (s, 3H, $COCH_3$), 2.40—3.00 (m, 8H), 3.46 (d, J=14, 1H, C$\underline{H}_2$Ph), 3.70 (d, J=14, 1H, C$\underline{H}_2$Ph), 4.01 (m, 1H, 6-CH), 6.43 (m, 1H, NHCO), 6.73—7.43 (m, 4H, arom H).

6-(Acetylamino)-1-(4-fluorobenzyl)-4-methylhexahydro-1*H*-1,4-diazepine (32c) In a similar manner to that described for the preparation of **32a**, compound **32c** was prepared from **31** and 4-fluorobenzyl chloride. ^{1}H-NMR (80 MHz, $CDCl_3$) δ: 1.90 (s, 3H, NCH_3), 2.46 (s, 3H, $COCH_3$), 2.21—2.90 (m, 8H), 3.45 (d, J=14, 1H, C$\underline{H}_2$Ph), 3.66 (d, J=14, 1H, C$\underline{H}_2$Ph), 4.02 (m, 1H, 6-CH), 6.40 (m, 1H, NHCO), 6.86—7.36 (m, 4H, arom H).

1-(Benzylamino)-3-(methylamino)-2-propanol (35) 1-(Benzylamino)-3-chloro-2-propanol[37] (**34**, 22.5 g, 0.11 mol) was added portionwise to a 30% solution of NH_2CH_3 in EtOH (100 ml) at room temperature. The mixture was heated at 50 °C for 16 h and then concentrated to dryness. A 40% aqueous KOH solution (30 ml) was added to the residue, and the mixture was extracted with $CHCl_3$. The extract was evaporated to leave an oil, which was distilled to give 7.0 g (32%) of **35**, bp 134—136 °C (1 mmHg). Compound **35** was converted to the dihydrochloride in the usual manner, mp 166—167 °C (EtOH). ^{1}H-NMR [200 MHz, dimethylsulfoxide (DMSO)-d_6] δ: 2.55 (s, 3H, NCH_3), 2.82—3.21 (m, 4H), 4.18 (s, 2H, CH_2Ph), 4.30 (m, 1H, 2-CH), 6.28 (d, J=5, 1H, OH), 7.38—7.70 (m, 5H, arom H), 8.6—9.9 (br s, 2H). *Anal.* Calcd for $C_{11}H_{18}N_2O \cdot 2HCl$: C, 49.45; H, 7.54; Cl, 26.54; N, 10.48. Found: C, 49.61; H, 7.32; Cl, 26.65; N, 10.40.

1-Benzyl-4-methylhexahydro-1*H*-1,4-diazepin-6-ol (36) A mixture of **35** · 2HCl (20.0 g, 75 mmol), 40% glyoxal in water (21.7 g, 0.15 mol), Et_3N (15.1 g, 0.15 mol), AcOH (3 drops), and MeOH (200 ml) was stirred at room temperature in the presence of platinum dioxide (1.0 g) under hydrogen. When hydrogen consumption ceased, the platinum dioxide was filtered off. The filtrate was concentrated to dryness. The residue was dissolved in $CHCl_3$ and then washed successively with water and brine. The solvent was evaporated to leave a crude product, which was chromatographed on silica gel with $CHCl_3$: MeOH = 50 : 1 to give 7.9 g (48%) of **36** as an oil. ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 2.39 (s, 3H, NCH_3), 2.25—2.85 (m, 8H), 3.68 (s, 2H, CH_2Ph), 3.77 (m, 1H, 6-CH), 7.20—7.36 (m, 5H, arom H). MS *m/z*: 221 (MH^+).

4-Amino-5-chloro-2-(*n*-propoxy)benzoic Acid (41a) A mixture of methyl 4-(acetylamino)-2-hydroxybenzoate[28a] (**37**, 5.2 g, 25 mmol), anhydrous K_2CO_3 (6.9 g, 50 mmol), *n*-propyl iodide (8.3 g, 50 mmol), and DMF (25 ml) was heated at 90 °C for 5 h and then cooled to room temperature. The reaction mixture was concentrated to dryness, dissolved in water, and extracted with $CHCl_3$. The extract was washed successively with water and brine. The solvent was evaporated to give a pale brown oil containing methyl 4-(acetylamino)-2-(*n*-propoxy)benzoate (**38a**). A mixture of the crude **38a**, NCS (3.4 g, 25 mmol), and DMF (15 ml) was heated at 80 °C for 4 h and poured into ice-water. The resulting precipitates were collected, washed with water, dried, and recrystallized from EtOH to give 4.4 g [62% yield from methyl 4-(acetylamino)-2-hydroxybenzoate] of methyl 4-(acetylamino)-5-chloro-2-(*n*-propoxy)benzoate (**39a**), mp 105—106 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 1.07 (t, J=7.5, 3H, $OCH_2CH_2C\underline{H}_3$), 1.86 (sex, J=7.5, 2H, $OCH_2C\underline{H}_2CH_3$), 2.26 (s, 3H, $NHCOC\underline{H}_3$), 3.87 (s, 3H, $COOCH_3$), 4.03 (t, J=7.5, 2H, $OC\underline{H}_2CH_2CH_3$), 7.76 (br s, 1H, $N\underline{H}COCH_3$), 7.87 (s, 1H, arom 3-H), 8.28 (s, 1H, arom 6-H). *Anal.* Calcd for $C_{13}H_{16}ClNO_4$: C, 54.65; H, 5.64; Cl, 12.41; N, 4.90. Found: C, 54.41; H, 5.57; Cl, 12.13; N, 4.88. IR (KBr) ν cm^{-1}: 3320, 2940, 1720, 1668, 1570, 1395, 1225. MS *m/z*: 286 (MH^+).

A mixture of **39a** (3.5 g, 12 mmol), 1 N aqueous NaOH (35 ml), and MeOH (25 ml) was heated to reflux for 4 h and then cooled to 5 °C. The reaction mixture was acidified with 25% aqueous H_2SO_4. The resulting precipitates were collected, washed with water, dried, and recrystallized from EtOH–*n*-hexane to afford 2.7 g (96%) of **41a**, mp 147—149 °C. ^{1}H-NMR (200 MHz, DMSO-d_6) δ: 0.98 (t, J=7.5, 3H, $OCH_2CH_2C\underline{H}_3$), 1.72 (sex, J=7.5, 2H, $OCH_2C\underline{H}_2CH_3$), 3.87 (t, J=7.5, 2H, $OC\underline{H}_2CH_2CH_3$), 6.05 (s, 2H, NH_2), 6.44 (s, 1H, arom 3-H), 7.59 (s, 1H, arom 6-H), 11.80 (s, 1H, COOH). *Anal.* Calcd for $C_{10}H_{12}ClNO_3$: C, 52.30; H, 5.27; Cl, 15.44; N, 6.10. Found: C, 52.29; H, 5.16; Cl, 15.37; N, 6.11. IR (KBr) ν cm^{-1}: 3460, 3290, 3225, 3180, 1695, 1605, 1575, 1430. MS *m/z*: 230 (MH^+), 212.

4-Amino-5-chloro-2-(iso-propoxy)benzoic Acid (41b) In a similar manner to that described above, compound **41b** was prepared from methyl 4-(acetylamino)-2-hydroxybenzoate and isopropyl iodide. Yield and spectral data of the intermediate **39b** and **41b** are given below.

Compound **39b**: 65%, mp 102—103 °C (EtOH). ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 1.36 (s, 3H, $OCH(C\underline{H}_3)_2$), 1.40 (s, 3H, $OCH(C\underline{H}_3)_2$), 2.26 (s, 3H, $NHCOC\underline{H}_3$), 3.85 (s, 3H, $COOCH_3$), 4.64 (hep, J=7.5, 1H, $OC\underline{H}(CH_3)_2$), 7.75 (br s, 1H, $N\underline{H}COCH_3$), 7.85 (s, 1H, arom 3-H), 8.29 (s, 1H, arom 6-H). *Anal.* Calcd for $C_{13}H_{16}ClNO_4$: C, 54.65; H, 5.64;

Cl, 12.41; N, 4.90. Found: C, 54.85; H, 5.57; Cl, 12.24; N, 4.91. IR (KBr) ν cm^{-1}: 3320, 2970, 1715, 1690, 1565, 1385, 1210. MS *m/z*: 286 (MH^+), 244.

Compound **41b**: 97%, mp 133—135 °C (EtOH–*n*-hexane). ^{1}H-NMR (200 MHz, DMSO-d_6) δ: 1.25 (s, 3H, OCH(C$\underline{H}_3$)$_2$), 1.28 (s, 3H, OCH(C$\underline{H}_3$)$_2$), 4.44 (hep, J=7.5, 1H, OC$\underline{H}$(CH$_3$)$_2$), 6.02 (s, 2H, NH_2), 6.46 (s, 1H, arom 3-H), 7.59 (s, 1H, arom 6-H), 11.94 (s, 1H, COOH). *Anal.* Calcd for $C_{10}H_{12}ClNO_3$: C, 52.30; H, 5.27; Cl, 15.44; N, 6.10. Found: C, 52.17; H, 5.23; Cl, 15.47; N, 6.05. IR (KBr) ν cm^{-1}: 3470, 3320, 3200, 2965, 1695, 1610, 1575, 1430. MS *m/z*: 230 (MH^+), 188.

1-Benzyl-*N*-(5-chloro-2-methoxyphenyl)-4-methylhexahydro-1*H*-1,4-diazepine-6-carboxamide (46) A mixture of 5-chloro-2-methoxyaniline (**44**, 4.6 g, 29 mmol), 1-benzyl-4-methylhexahydro-1*H*-1,4-diazepine-6-carboxylic acid dihydrochloride[39] (**45**, 9.3 g, 29 mmol), WSC (6.7 g, 35 mmol), and CH_2Cl_2 (250 ml) was stirred at room temperature for 15 h. The reaction mixture was washed successively with water, 10% aqueous NaOH, water, and brine, and concentrated to dryness. The residue was crystallized from iso-PrOH to give 7.0 g (62%) of **46**, mp 99—100 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 2.42 (s, 3H, NCH_3), 2.38—2.88 (m, 5H), 2.93 (d, J=4, 2H), 3.04 (dd, J=4, 6, 2H), 3.63 (d, J=13.5, 1H, C$\underline{H}_2$Ph), 3.72 (d, J=13.5, 1H, C$\underline{H}_2$Ph), 3.79 (s, 3H, OCH_3), 6.77 (d, J=8.0, 1H, arom 3-H), 6.98 (dd, J=2.0, 8.0, 1H, arom 4-H), 7.17—7.32 (m, 5H, arom H), 8.46 (d, J=2.0, 1H, arom 6-H), 10.86 (br s, 1H, NHCO). *Anal.* Calcd for $C_{21}H_{26}ClN_3O_2$: C, 65.02; H, 6.76; Cl, 9.14; N, 10.89. Found: C, 65.08; H, 6.79; Cl, 9.21; N, 10.76. IR (KBr) ν cm^{-1}: 2920, 2800, 1660, 1580, 1520. MS *m/z*: 388 (MH^+).

1-Benzyl-*N*-(5-chloro-2-methoxy-4-nitrophenyl)-4-methylhexahydro-1*H*-1,4-diazepine-6-carboxamide (47) Fuming HNO_3 (d=1.50, 1.0 ml) was added dropwise to a solution of **46** (7.8 g, 20 mmol) in a mixture of AcOH (50 ml) and concentrated H_2SO_4 (2.5 ml) at room temperature. The mixture was stirred at room temperature for 3 h and then poured into ice-water. The solution was basified with 48% aqueous NaOH and extracted with $CHCl_3$. The extract was washed with brine and concentrated to dryness. The residue was crystallized from EtOH to give 7.7 g (88%) of **47**, mp 118—121 °C. ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 2.43 (s, 3H, NCH_3), 2.38—2.88 (m, 5H), 2.93 (t, J=3, 2H), 3.05 (d, J=4, 2H), 3.63 (d, J=13.5, 1H, C$\underline{H}_2$Ph), 3.70 (d, J=13.5, 1H, C$\underline{H}_2$Ph), 3.91 (s, 3H, OCH_3), 7.22 (s, 5H, arom H), 7.55 (s, 1H, arom 3-H), 8.73 (s, 1H, arom 6-H), 11.64 (br s, 1H, NHCO). *Anal.* Calcd for $C_{21}H_{25}ClN_4O_4$: C, 58.26; H, 5.82; Cl, 8.19; N, 12.94. Found: C, 58.42; H, 5.78; Cl, 8.33; N, 12.92. IR (KBr) ν cm^{-1}: 2900, 2800, 1670, 1565, 1520, 1325. MS *m/z*: 433 (MH^+).

General Procedure for the Preparation of the Benzamide Derivatives (58—63, 65—69, 71, 75—77, 79, 81—84, 87, 94—102, 104—110, 116—118, 120—129) The acetylamino derivatives (**10c**, **15a**, **b**, **16a**, **b**, **20**, **29**, **32a**—**c**) were hydrolyzed with 10% HCl to give the corresponding amines, which were used in the next step without further purification.

A mixture of benzoic acid (10 mmol), amine (10 mmol), WSC (12 mmol), and CH_2Cl_2 (80 ml) was stirred at room temperature for 5 h. The reaction mixture was washed successively with water, 10% aqueous NaOH, water, and brine. The solvent was evaporated to leave a crude product, which was chromatographed on silica gel. The product was recrystallized from the solvent given Tables 3—5 and 7 or converted to the fumarate or oxalate in the usual manner, followed by recrystallization from the solvent given in Tables 3—5 and 7.

General Procedure for the Preparation of the 1,4-Diazepinyl Esters (111—114) A mixture of 2-alkoxy-4-amino-5-chlorobenzoic acid (**40a** or **40b**, 7.0 mmol), the 1,4-diazepin-6-ol **33** or **36** (6.9 mmol), WSC (7.3 mmol), DMAP (0.4 g), and CH_2Cl_2 (70 ml) was stirred at room temperature for 15 h. The reaction mixture was washed successively with water, 10% aqueous NaOH, water, and brine. The solvent was evaporated to leave a crude product, which was chromatographed on silica gel with $CHCl_3$: MeOH = 9 : 1. The products were recrystallized (**111**, **113**) and converted to the fumarate (**112**) or the hydrochloride (**114**) in the usual manner, followed by recrystallization from the solvent given in Table 6.

***cis*- and *trans*-4-Amino-*N*-[(4-benzyl-5-methyl-2-morpholinyl)methyl]-5-chloro-2-ethoxybenzamides (59, 60)** The mixture of compounds **59** and **60** was separated by silica gel column chromatography (eluent: $CHCl_3$) to give **59** (47%) as an amorphous solid and **60** (37%) as a solid in that order. Compound **59** was converted to the fumarate in the usual manner.

Compound **59** (Base): ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.09 (d, J=6.6, 3H, 5-CH_3), 1.48 (t, J=7.5, 3H, $OCH_2C\underline{H}_3$), 2.43 (dd, J=3.8, 11.8, 1H, 3-H_{eq}), 2.48 (dd, J=8.1, 11.8, 1H, 3-H_{ax}), 2.77 (ddd, J=2.9, 6.6, 13.8, 1H, 5-H), 3.45 (m, 1H, CONHC$\underline{H}_2$), 3.51 (d, J=13.3, 1H, C$\underline{H}_2$Ph), 3.61 (d, J=13.3, 1H, C$\underline{H}_2$Ph), 3.62 (m, 1H, CONHC$\underline{H}_2$), 3.67 (dd, J=11.0, 13.8, 1H, 6-H_{ax}), 3.78 (dd, J=2.9, 11.0, 1H, 6-H_{eq}), 3.71 (dddd, J=3.6, 3.8, 8.1, 8.2, 1H, 2-H), 4.39 (q, J=7.5, 2H, $OC\underline{H}_2CH_3$), 4.24 (s, 2H, NH_2), 6.28 (s, 1H, arom 3-H), 7.20—7.40 (m, 5H), 8.13 (s, 1H, arom 6-H), 8.24 (br t, 1H, NHCO). IR (KBr) ν cm^{-1}: 3470, 3390, 3320, 3200, 2975, 1630, 1585, 1520, 1495. MS *m/z*: 418 (MH^+).

Compound **60**: ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.08 (d, J=6.6, 3H, 5-CH_3), 1.46 (t, J=7.5, 3H, $OCH_2C\underline{H}_3$), 1.93 (dd, J=10.1, 11.6, 1H, 3-H_{ax}), 2.41 (m, 1H, 5-H), 2.67 (dd, J=2.1, 11.6, 1H, 3-H_{eq}), 3.06 (d, J=13.3, 1H, C$\underline{H}_2$Ph), 3.22 (m, 1H, CONHC$\underline{H}_2$), 3.34 (dd, J=10.3, 11.2, 1H, 6-H_{ax}), 3.63 (m, 1H, CONHC$\underline{H}_2$), 3.64 (m, 1H, 2-H), 3.76 (dd, J=3.3, 11.2, 1H, 6-H_{eq}), 4.04 (q, J=7.5, 2H, $OC\underline{H}_2CH_3$), 4.12 (d, J=13.3, 1H, C$\underline{H}_2$Ph), 4.32 (s, 2H, NH_2), 6.24 (s, 1H, arom 3-H), 7.15—7.35 (m, 5H), 8.07 (s, 1H, arom 6-H), 8.15 (br t, 1H, NHCO). IR (KBr) ν cm^{-1}: 3455, 3375, 3300, 3280, 1630, 1580, 1530, 1495. MS *m/z*: 418 (MH^+).

4-Amino-5-chloro-*N*-[(1,4-diethyl-2-piperazinyl)methyl]-2-ethoxybenzamide Fumarate (83) and 4-Amino-5-chloro-*N*-(1,4-diethylhexahydro-1*H*-1,4-diazepin-6-yl)-2-ethoxybenzamide Fumarate (101) The mixture of the free base of **83** and **101** was separated by medium-pressure silica gel column chromatography (eluent: acetone) to give 4-amino-5-chloro-*N*-[(1,4-diethyl-2-piperazinyl)methyl]-2-ethoxybenzamide and 4-amino-5-chloro-*N*-(1,4-diethylhexahydro-1*H*-1,4-diazepin-6-yl)-2-ethoxybenzamide in 65% and 7% yields, respectively, from **14b**. Each compound was converted to the fumarate in the usual manner.

Compound **83**: ^{1}H-NMR (200 MHz, DMSO-d_6) δ: 0.98 (t, J=7, 3H, $NCH_2C\underline{H}_3$), 1.00 (t, J=7, 3H, $NCH_2C\underline{H}_3$), 1.43 (t, J=7, 3H, $OCH_2C\underline{H}_3$), 2.03—2.27 (m, 2H), 2.27—2.68 (m, 5H), 2.68—2.93 (m, 4H), 3.39—3.55 (m, 2H), 4.09 (q, J=7, 2H, $OC\underline{H}_2CH_3$), 5.94 (s, 2H, NH_2), 6.49 (s, 1H, arom 3-H), 6.68 (s, 2H), 7.73 (s, 1H, arom 6-H), 8.06 (br t, J=7, CONH). MS *m/z*: 369 (MH^+), 198, 170.

Compound **101**: ^{1}H-NMR (200 MHz, DMSO-d_6) δ: 0.97 (t, J=7, 6H, $NCH_2C\underline{H}_3$ × 2), 1.42 (t, J=7, 3H, $OCH_2C\underline{H}_3$), 2.5—2.95 (m, 12H), 4.09 (q, J=7, 2H, $OC\underline{H}_2CH_3$), 4.15 (m, 1H, 6-H), 5.93 (s, 2H, NH_2), 6.47 (s, 1H, arom 3-H), 6.60 (s, 3H), 7.74 (s, 1H, arom 6-H), 8.42 (d, J=7, CONH). MS *m/z*: 369 (MH^+), 198, 170.

4-Amino-5-chloro-*N*-[(1,4-diethyl-2-piperazinyl)methyl]-2-methoxybenzamide Fumarate (84) and 4-Amino-5-chloro-*N*-(1,4-diethylhexahydro-1*H*-1,4-diazepin-6-yl)-2-methoxybenzamide Fumarate (102) In a similar manner to that described above, compounds **84** and **102** were separated in 66% and 7% yields, respectively, from **14b**. Each compound was converted to the fumarate in the usual manner.

7-Amino-*N*-(1-benzyl-4-methylhexahydro-1*H*-1,4-diazepin-6-yl)-6-chloro-4*H*-1,3-benzoxazin-2,4-dione (119) Ethanethiol (2.9 g, 47 mmol) was added to a suspension of sodium hydride (60% dispersion in mineral oil, 1.9 g, 48 mmol) in anhydrous DMF (60 ml) under ice-cooling. The reaction mixture was stirred at room temperature for 0.5 h, and then the free base of **104** (6.3 g, 16 mmol) was added. The whole was heated to reflux for 3 h and concentrated to dryness. The residue was taken up in water and washed with $CHCl_3$. The aqueous layer was neutralized with 10% HCl and extracted with $CHCl_3$. The extract was washed with brine and evaporated. The residue was chromatographed on silica gel with $CHCl_3$: MeOH = 30 : 1 to give 5.0 g (82%) of 4-amino-*N*-(1-benzyl-4-methylhexahydro-1*H*-1,4-diazepin-6-yl)-5-chloro-2-hydroxybenzamide (**43**) as an oil [MS *m/z*: 389 (MH^+)].

A solution of **43** (1.0 g, 2.6 mmol) and Et_3N (2.6 g, 26 mmol) in $CHCl_3$ (100 ml) was treated with $COCl_2$ (30% solution in toluene, 0.5 g, 3.0 mmol) at room temperature. The mixture was stirred at room temperature for 15 h and concentrated to dryness. The residue was chromatographed on silica gel with $CHCl_3$: MeOH = 50 : 1 to give a solid, which was recrystallized from EtOH to afford 0.6 g (56%) of **119**. ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 2.48 (s, 3H, NCH_3), 2.55—2.96 (m, 6H), 3.40—3.62 (m, 2H), 3.68 (d, J=11, 1H, C$\underline{H}_2$Ph), 3.80 (d, J=11, 1H, C$\underline{H}_2$Ph), 4.86 (s, 2H, NH_2), 5.35 (m, 1H, 6-H), 6.50 (s, 1H, arom 8-H), 7.16—7.41 (m, 5H, arom H), 7.90 (s, 1H, arom 5-H). MS *m/z*: 415 (MH^+).

7-Amino-*N*-[(4-benzyl-2-morpholinyl)methyl]-6-chloro-4*H*-1,3-benzoxazin-2,4-dione (64) In a similar manner to that described above, compound **64** was prepared from 4-amino-*N*-[(4-benzyl-2-morpholinyl)methyl]-5-chloro-2-hydroxybenzamide[29] (**42**) in 50% yield. ^{1}H-NMR (80 MHz, $CDCl_3$) δ: 1.91—2.92 (m, 4H), 3.49 (s, 2H, NH_2), 3.52—4.51 (m, 5H), 4.80 (s, 2H, CH_2Ph), 6.50 (s, 1H, arom 8-H), 7.25

(s), 7.30 (s, 5H, arom H), 7.91 (s, 1H, arom 5-H). IR (KBr) ν cm^{-1}: 1750, 1670. MS *m/z*: 402 (MH^+).

3-(4-Amino-5-chloro-2-methoxybenzamidomethyl)-4-benzyltetrahydro-4*H*-1,4-thiazin 1-Oxide (88) *m*-CPBA (0.47 g, 2.7 mmol) was added to a solution of **87** (1.1 g, 2.7 mmol) in CH_2Cl_2 (20 ml) dropwise at −20 °C. The mixture was stirred at the same temperature for 45 min. The solution was washed successively with saturated aqueous $NaHCO_3$, water, and brine, and then concentrated to dryness. The residue was chromatographed on silica gel with $CHCl_3$: MeOH = 40 : 1 to afford 0.6 g (52%) of **88**. ^{1}H-NMR (80 MHz, $CDCl_3$) δ: 2.52—4.05 (m, 11H), 3.90 (s, 3H, OCH_3), 4.46 (s, 2H, CH_2Ph), 6.32 (s, 1H, arom 3-H), 7.29 (s, 5H, arom H), 8.05 (s, 1H, arom 6-H), 7.9—8.3 (m, 1H, CONH). IR (KBr) ν cm^{-1}: 1635, 1615.

***N*-(4-Amino-5-chloro-2-methoxyphenyl)-1-benzyl-4-methylhexahydro-1*H*-1,4-diazepine-6-carboxamide (115)** A solution of stannous chloride dihydrate (19.5 g, 86 mmol) in concentrated HCl (40 ml) was added dropwise to a solution of **47** (12.5 g, 29 mmol) in AcOH (80 ml) at 10 °C. The mixture was stirred at room temperature for 20 h and then poured into ice-water. The solution was basified with 10% aqueous NaOH and extracted with $CHCl_3$. The extract was washed with brine and evaporated. The residue was crystallized to afford 8.5 g (76%) of **115**. ^{1}H-NMR (200 MHz, $CDCl_3$) δ: 2.41 (s, 3H, NCH_3), 2.40—2.85 (m, 5H), 2.92 (d, J=4, 2H), 3.05 (dd, J=4, 6, 2H), 3.63 (d, J=13.5, 1H, C$\underline{H}_2$Ph), 3.72 (d, J= 13.5, 1H, C$\underline{H}_2$Ph), 3.73 (s, 3H, OCH_3), 3.91 (s, 2H, NH_2), 6.32 (s, 1H, arom 3-H), 7.20—7.45 (m, 5H, arom H), 8.28 (s, 1H, arom 6-H), 10.41 (br s, 1H, NHCO). IR (KBr) ν cm^{-1}: 1620, 1585, 1520. MS *m/z*: 403 (MH^+).

Biological Activities Male rats of the JCL SD strain (Nihon SLC Inc., Shizuoka, Japan) weighing 300—350 g, and male albino ferrets (Marshall Res. Animal Inc., N.Y., U.S.A.) weighing 1—1.5 kg were used. The compounds prepared were dissolved in saline at room temperature, and cisplatin was dissolved in saline at 70 °C.

B-J Reflex (2-Methyl-5-HT-Induced Bradycardia) Rats were anesthetized with urethane (1.2 g/kg, i.p.). The heart rate was derived from the electrocardiogram (lead II), which was recorded *via* electrodes s.c. inserted into the left forelimb and right hindlimb. The femoral vein was cannulated for i.v. injection of 2-methyl-5-HT and test compounds. Bolus i.v. injections of 2-methyl-5-HT (30—50 μg/kg) were given every 15 min. After the 2-methyl-5-HT-induced bradycardia had become stable, a test compound was injected i.v. 3 min before administration of 2-methyl-5-HT. The ED_{50} values of compounds (dose causing 50% inhibition of the bradycardia) were obtained by Probit analysis.[51]

Cisplatin-Induced Emesis in Ferrets Under pentobarbital anesthesia (30 mg/kg, i.p.), a chronic indwelling jugular venous catheter was surgically implanted for i.v. injection of cisplatin and test compounds in ferrets, as reported by Florczyk and Schurig.[52] Two to three days after operation, test compounds were administered i.v. twice at 30 min before and 45 min after administration of cisplatin (10 mg/kg, i.v.). The latency from administration of cisplatin to the first emetic episode and the number of emetic episodes induced were observed for 3 h after administration of cisplatin. Differences from the control group that were statistically significant were identified by means of the MUSCOT statistical analysis program (Yukms Co., Tokyo, Japan; Williams–Wilcoxon's multiple range test).

5-HT$_3$ Receptor Binding Assay[53] The binding assay was carried out according to the method described in the previous paper. Male Std-Wistar rats (200—250 g) were decapitated. The frontal cortex was dissected and homogenized in 10 volumes of ice-cold 0.32 M sucrose in a Potter-Elvehjem glass homogenizer fitted with a Teflon pestle. The homogenate was centrifuged at 1000 × *g* for 10 min and the pellet was discarded. The supernatant was centrifuged at 17200 × *g* for 10 min. The crude mitochondrial pellet was resuspended in 20 volumes of Krebs–HEPES buffer and centrifuged at 17200 × *g* for 10 min. The pellet (fraction P_2) was resuspended in 20 volumes of Krebs–HEPES buffer containing 0.01—0.1% Triton X-100. After a 30-min incubation at 37 °C, the membranes were washed twice by recentrifugation (50000 × *g* for 15 min) and resuspension in Triton X-100-free buffer (Krebs–HEPES buffer). The pellet was finally suspended in 40 volumes of the same buffer. A Krebs–HEPES buffer consisting of 25 mM HEPES, 180 mM NaCl, 5 mM KCl, 2.5 mM $CaCl_2$, and 1.2 mM $MgCl_2$ (pH adjusted to 7.4) was used. Krebs–HEPES buffer (500 μl) with or without the drug, was added to assay tubes and 100 vl of [^{3}H]quipazine was added at final concentrations of 1.0 mM and 0.3—4.0 mM for drug competition and saturation studies, respectively. Subsequently, the membrane suspension (0.2 mg protein/400 μl) was added to initiate binding. The assay tubes were incubated for 30 min at 25 °C. The incubation was terminated by rapid filtration under reduced pressure through Whatman GF/B filters presoaked in 0.03% polyethylenimine. The filters were immediately washed three times with 4 ml of ice-cold 50 mM Tris–HCl buffer (pH 7.7). All experiments were performed in duplicate or triplicate. Radioactivity was measured by liquid scintillation counting in 10 ml of ACS II scintillator (Amersham).

5-HT$_4$ Receptor Binding Assay The binding assay was carried out according to the method of Grossman *et al.*[54] All determinations were performed in triplicate. Assay tubes contained 300 μl of HEPES buffer at pH 7.4, 200 μl of a solution of either a competing agent (for drug competition studies), 5-HT to give a final concentration of 30 μM (to determine non-specific binding) or buffer (for determination of total binding), and 400 μl of [^{3}H]GR113808 in HEPES buffer to give a final concentration of 0.1 nM and 100 μl of tissue preparation. Assay tubes were incubated at 37 °C. The reaction was terminated by rapid vacuum filtration and washing (1 × 4 ml) with ice-cold buffer through Whatman GF/B filter paper using a Brandel Cell Harvester. Filters were presoaked in a solution of polyethylenimine (*ca.* 0.1%) to reduce filter binding. For drug competition studies, assay tubes were incubated at 37 °C for 30 min and the reaction was terminated as above. Filters were placed in 10 ml of ACS II scintillator (Amersham) before scintillation counting.

Acknowledgment The authors thank the staff of the Analytical Chemistry Division of the Exploratory Research Laboratories, Dainippon Pharmaceutical Company, for elemental analyses and spectral measurements.

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