

Design and Synthesis of 6-Chloro-3,4-dihydro-4-methyl-2H-1,4-benzoxazine-8-carboxamide Derivatives as Potent Serotonin-3 (5-HT₃) Receptor Antagonists

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Several 3-substituted 5-chloro-2-methoxybenzamides were synthesized and evaluated for serotonin-3 (5-HT₃) receptor binding affinity. The 5-HT₃ receptor antagonistic activity of zacopride, a representative 5-HT₃ receptor antagonist, was unchanged by the replacement of the 4-amino substituent on the aromatic moiety by a 3-dimethyl-amino substituent. This finding prompted a structural modification of azasetron, another 5-HT₃ receptor antagonist. Consequently, a new series of 3,4-dihydro-2H-1,4-benzoxazine-8-carboxamides was obtained and these compounds were found to be more potent than 3,4-dihydro-3-oxo-2H-1,4-benzoxazine-8-carboxamides. In particular, (*S*)-*N*-(1-azabicyclo[2.2.2]oct-3-yl)-6-chloro-3,4-dihydro-4-methyl-2H-1,4-benzoxazine-8-carboxamide showed a high affinity for 5-HT₃ receptors ($K_i = 0.051$ nM) and especially potent antagonistic activity against the von Bezold-Jarisch reflex ($ED_{50} = 0.089$ µg/kg i.v.) in rats.

Key words 1,4-benzoxazine-8-carboxamide; 5-HT₃ receptor antagonist; structure–activity relationship; 5-HT₃ receptor binding; von Bezold-Jarisch reflex

Serotonin (5-HT) exerts a wide variety of behavioral and physiological effects through actions on multiple receptor subtypes. Pharmacological and molecular cloning approaches have identified at least fourteen distinct subtypes of mammalian 5-HT receptors, which have been classified into seven families with unique structural, transductional and operational characteristics. The 5-HT receptors mostly belong to a G-protein linked receptor superfamily.^{1a)} The 5-HT₃ receptors, however, are not related to G-proteins but belong to a ligand-gated ion channel superfamily.^{1a–d)} Medicinal chemists are interested in 5-HT₃ receptor antagonists, because 5-HT₃ receptors have been identified in the peripheral and central nervous systems.^{2a–d)} Following the discovery of various 5-HT₃ receptor antagonists, binding models for these compounds have been presented by three research groups.^{3a–c)} They proposed three key pharmacophoric elements, which were an aromatic moiety, a carbonyl function or a bioisosteric group, and a basic nitrogen atom. Most 5-HT₃ receptor antagonists so far known have such a pharmacophore. Such antagonists can be regarded as falling into two classes based on the structure of the aromatic moiety. One is an indole family, which includes indole-3-carboxamides, indole-3-carboxylates and (indole-3-yl)methanone derivatives, as well as indazole-3-carboxamides such as granisetron.⁴⁾ The other is a benzamide family.

Zacopride⁵⁾ is a typical member of the benzamide family. The 4-aminobenzamide structure has been reported to play an important role in the 5-HT₃ receptor antagonistic activity of zacopride.⁶⁾ It has been claimed that the benzamide family can be superimposed on the indole family by use of computer modeling.^{3a,b)} Nevertheless, the arrangement of nuclear nitrogen and the carbonyl function in the indole family may be comparable with that of the amino nitrogen and carbonyl function in 3-aminobenzamide, rather than that in 4-aminobenzamide. We therefore chose zacopride as a tentative lead compound

for structural modification, and transferred the amino moiety from position 4 to position 3 (compounds **7a–c**). The synthesis of the 3-aminobenzamide derivatives **7a–c** might provide new, potent 5-HT₃ receptor antagonists and should provide additional information on the structural properties of the pharmacophore. On the basis of the results, we carried out some modification of azasetron,⁷⁾ another member of the benzamide family, and prepared 3,4-dihydro-2H-1,4-benzoxazine-8-carboxamide derivatives **13a–j** and **17a–f**. In this paper we will report the synthesis and structure–activity relationships of 6-chloro-3,4-dihydro-2H-1,4-benzoxazine-8-carboxamide derivatives as new 5-HT₃ receptor antagonists.

Chemistry

3-Aminobenzamide derivatives **7a–c**, listed in Table 1, were prepared as shown in Chart 2. Commercially available 5-chloro-2-hydroxybenzoic acid was converted into ethyl 3-amino-5-chloro-2-hydroxybenzoate **1** in three steps as reported in the previous paper.⁷⁾ Methylation of **1** with iodomethane provided **2**, and then hydrolysis with base afforded the carboxylic acid **3a**. The *N*-acetyl compound **4** was obtained by acylation of **1** with acetyl chloride. *O*-Methylation of **4** with iodomethane provided **5**, which was hydrolyzed with base to provide **3b**. Compound **6** was prepared from **5** by *N*-methylation of the acetamide moiety, followed by hydrolysis to provide **3c**. The 3-aminobenzamide derivatives **7a–c** were prepared from the corresponding carboxylic acids **3a–c** by coupl-

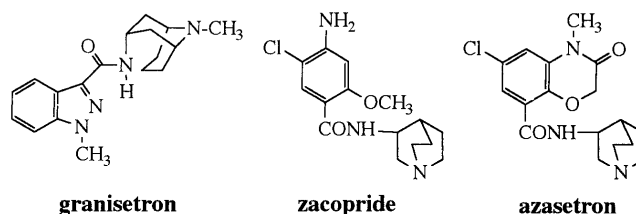


Chart 1

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ing with 3-amino-1-azabicyclo[2.2.2]octane *via* mixed anhydrides.

The general route for the synthesis of the 1,4-benzoxazine-8-carboxamide derivatives **13a–j** is shown in Chart 3. Cyclization of ethyl 3-acetamido-5-chlorosalicylate **4** with 1,2-dibromoethane provided **8** with the desired ring system. Alkaline hydrolysis of **8** under reflux gave **9**, followed by esterification to provide the key intermediate **10**. The 4-unsubstituted compound **13a** was prepared from the carboxylic acid **9** by coupling with 3-amino-1-azabicyclo[2.2.2]octane *via* the mixed anhydride. 4-Methylation of intermediate **10** with iodomethane provided **11b** in 80% yield. 4-Alkylation with other alkyl halides or aralkyl halides gave the desired compounds **11c–h** only in low yields, but these products were also obtained by reductive amination of **10** with the appropriate aldehyde in the presence of NaBH_3CN . The acylated compounds **11i–j** were obtained by acylation of

the intermediate **10** with the corresponding acyl halide. Compounds **11c–j**, used without further purification, were hydrolyzed with base and the products were purified by column chromatography on silica gel to give **12c–j**. The carboxylic acids **12c–j** were coupled with 3-amino-1-azabicyclo[2.2.2]octane to give **13c–j**.

The 6-substituted compounds **17a–d** were prepared as shown in Chart 4. Key intermediates **15a–d** were prepared from the amides **14a–d**⁷⁾ by Merkel's method,⁸⁾ which permits the selective reduction of the amide moiety in the presence of the ester moiety. Compounds **15a–d** were methylated at position 4 with iodomethane in the presence of K_2CO_3 , followed by hydrolysis with base to afford the carboxylic acids **16a–d**. Compounds **16a–d** were coupled with 3-amino-1-azabicyclo[2.2.2]octane to give **17a–d**. Compounds **17e** and **17f** were prepared as shown in Chart 5. The carboxylic acid **16a** was nitrated with fuming HNO_3 and H_2SO_4 followed by condensation

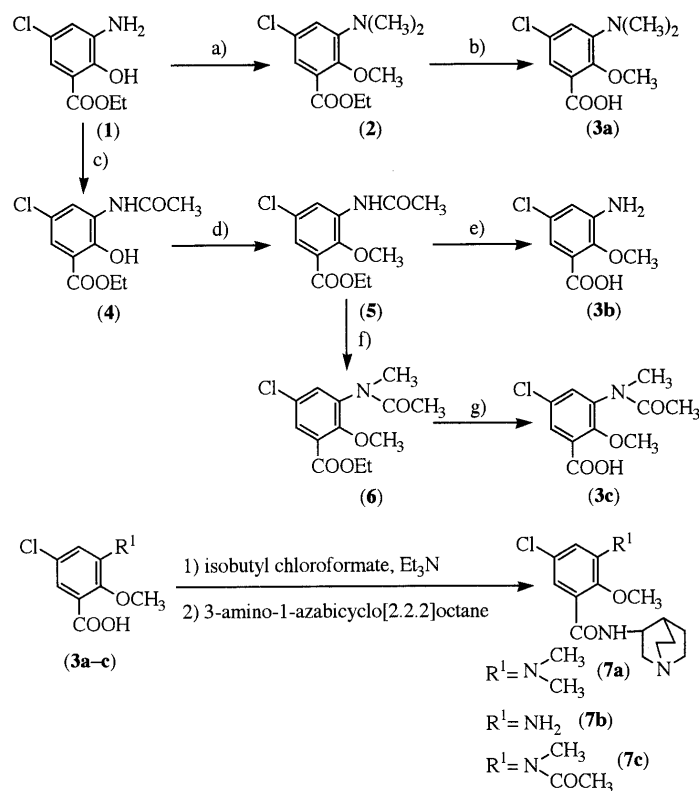
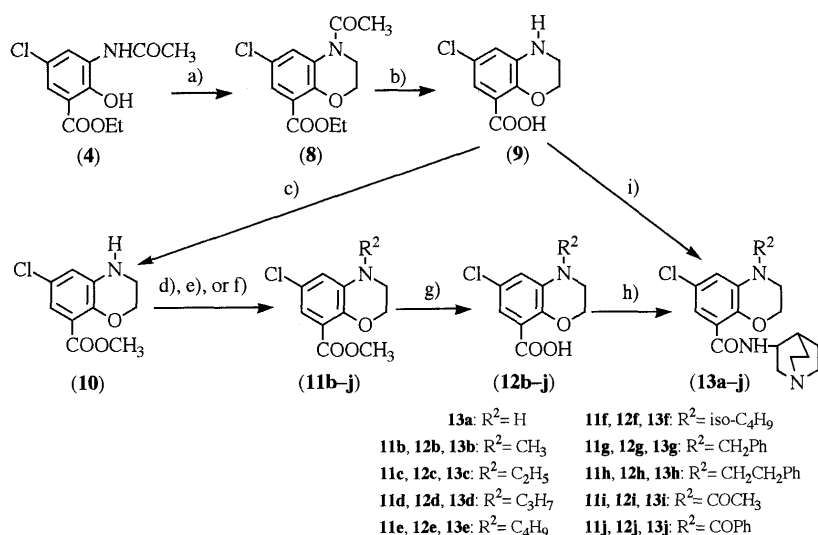


Chart 2

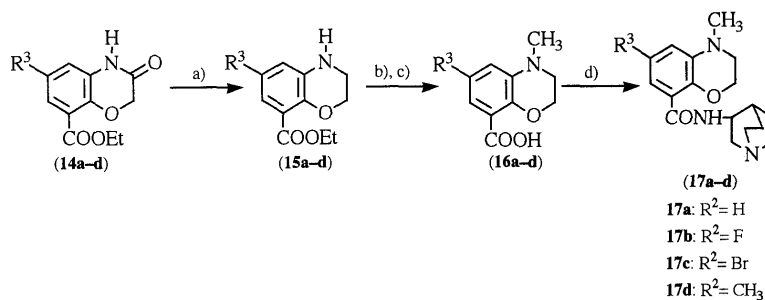
Table 1. 5-HT₃ Receptor Binding Affinity of Compounds **7a–c**

Compd. No.	mp (°C) (Recryst. solvent)	Yield (%)	Formula	Analysis (%)						^{[3]H} Granisetron binding K _i (nM)
				Calcd			Found			
				C	H	N	C	H	N	
7a	159—160 (AcOEt)	61.2	C ₁₇ H ₂₄ ClN ₃ O ₂	60.38	7.10	12.43	60.34	7.10	12.40	0.047
7b	192—194 (AcOEt)	25.8	C ₁₅ H ₂₀ ClN ₃ O ₂	58.16	6.51	13.56	57.91	6.62	13.72	3.7
7c	186—188 (EtOH–acetone)	60.0	C ₁₈ H ₂₄ ClN ₃ O ₃ ·oxalate	52.64	5.70	9.21	52.32	5.65	9.13	22
Zacopride										0.18



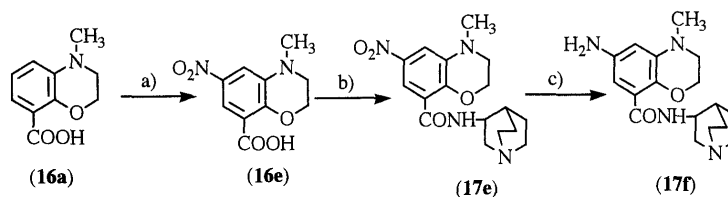
a) 1,2-dibromoethane, K₂CO₃; b) NaOH, 70°C; c) MeOH, H₂SO₄; d) CH₃I, K₂CO₃; e) RCHO, NaBH₃CN; f) RCOCl, Et₃N; g) NaOH, 50°C; h) pivaloyl chloride, Et₃N, racemate or enantiomers of 3-amino-1-azabicyclo[2.2.2]octane; i) isobutyl chloroformate, Et₃N, 3-amino-1-azabicyclo[2.2.2]octane

Chart 3



a) BF₃ · Et₂O, NaBH₄; b) CH₃I, K₂CO₃; c) NaOH, r.t.; d) pivaloyl chloride, Et₃N, 3-amino-1-azabicyclo[2.2.2]octane

Chart 4



a) HNO₃, H₂SO₄; b) pivaloyl chloride, Et₃N, 3-amino-1-azabicyclo[2.2.2]octane; c) Raney Ni, H₂

Chart 5

with the amine to give **17e**. The 6-amino compound **17f** was obtained by reduction of **17e**.

The enantiomers of **13b** shown in Table 5 were prepared by coupling the carboxylic acid **12b** with (*S*)- or (*R*)-3-amino-1-azabicyclo[2.2.2]octane.⁹ The reaction was performed by the mixed anhydride method under the conditions shown in Chart 3.

Pharmacological Results and Discussion

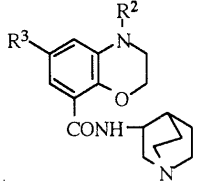
The 5-HT₃ receptor binding affinity of the synthesized compounds (**7a–c**, **13a–j** and **17a–f**) was determined by measurement of displacement of [³H]granisetron binding in rat cerebrocortical membranes.¹⁰ The 5-HT₃ receptor antagonistic activity was assessed in terms of the ability to inhibit 5-HT-induced bradycardia (von Bezold-

Jarisch reflex)¹¹ in rats, as shown in Table 4.

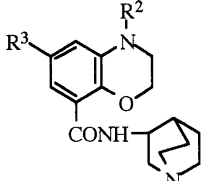
Compound **7a** (*K*_i = 0.047 nM) was 4-fold more potent than zacopride (*K*_i = 0.18 nM), although compounds **7b** and **7c** (*K*_i = 3.7 and 22 nM) showed less potent activity (Table 1). The 4-amino substituent on the aromatic moiety of zacopride is replaceable by a 3-dimethylamino substituent, but not by a simple 3-amino substituent. This result prompted us to modify the structure of azasetron, namely (±)-*N*-(1-azabicyclo[2.2.2]oct-3-yl)-6-chloro-3,4-dihydro-4-methyl-3-oxo-2*H*-1,4-benzoxazine-8-carboxamide, which is a potent and selective 5-HT₃ receptor antagonist and is marketed as an antiemetic. The nitrogen atom at position 4 of the 1,4-benzoxazine ring on azasetron corresponds to the 3-dimethylamino group on the aromatic moiety of compound **7a**. However, the nitrogen

atom at position 4 of the 1,4-benzoxazine ring of azasetron is never basic, because of its amido structure. Therefore, we intended to change the amido moiety into an amino moiety. This idea led us to design **13a–j**, in which the aromatic moiety is a 6-chloro-3,4-dihydro-2*H*-1,4-benzoxazine ring. As we expected, compound **13b** was

7.5-fold more potent than azasetron (Table 2) and was 2.5-fold more potent than zacopride (Table 1). Removal of the methyl group at position 4 resulted in reduced activity (**13a**, $K_i = 0.66$ nM). Replacement of the methyl group with larger substituents resulted in a reduction of the activity in the order of ethyl (**13c**) > normal-propyl

Table 2. 5-HT₃ Receptor Binding Affinity of Compounds **13a–j**


Compd. No.	R ²	R ³	mp (°C) (Recryst. solvent)	Yield (%)	Formula	Analysis (%) Calcd (Found)			[³ H]Granisetron binding K_i (nM)
						C	H	N	
13a	H	Cl	212–214 (EtOH)	19	C ₁₆ H ₂₀ ClN ₃ O ₂ ·2HCl·1/2H ₂ O	47.60 (47.73)	5.74 (5.47)	10.41 (10.10)	0.66
13b	CH ₃	Cl	162 (dec.) (EtOH–AcOEt)	72	C ₁₇ H ₂₂ ClN ₃ O ₂ ·2HCl·1/2H ₂ O	48.88 (48.99)	6.03 (5.98)	10.06 (9.73)	0.072
13c	CH ₂ CH ₃	Cl	209–211 (EtOH–acetone)	62	C ₁₈ H ₂₄ ClN ₃ O ₂ ·fumarate·1/4H ₂ O	56.17 (56.01)	6.11 (6.09)	8.93 (8.58)	0.3
13d	CH ₂ CH ₂ CH ₃	Cl	212–214 (EtOH–acetone)	65	C ₁₉ H ₂₆ ClN ₃ O ₂ ·fumarate	57.25 (57.56)	6.39 (6.30)	8.43 (8.75)	1.8
13e	CH ₂ CH ₂ CH ₂ CH ₃	Cl	193–194 (EtOH–acetone)	56	C ₂₀ H ₂₈ ClN ₃ O ₂ ·fumarate	57.98 (58.35)	6.52 (6.53)	8.41 (8.51)	1.8
13f	CH ₂ CH(CH ₃) ₂	Cl	204–206 (EtOH–acetone)	54	C ₂₀ H ₂₈ ClN ₃ O ₂ ·fumarate·1/4H ₂ O	57.83 (57.54)	6.57 (6.61)	8.43 (8.09)	6.8
13g	CH ₂ Ph	Cl	237–239 (EtOH)	38	C ₂₃ H ₂₆ ClN ₃ O ₂ ·fumarate·H ₂ O	59.39 (60.37)	5.91 (5.77)	7.70 (7.59)	3.5
13h	CH ₂ CH ₂ Ph	Cl	185–186 (EtOH)	60	C ₂₄ H ₂₈ ClN ₃ O ₂ ·fumarate·H ₂ O	60.05 (59.98)	6.12 (5.71)	7.50 (6.95)	7.4
13i	COCH ₃	Cl	155 (dec.) (EtOH)	34	C ₁₈ H ₂₂ ClN ₃ O ₃ ·HCl·2H ₂ O	49.55 (49.30)	6.24 (5.73)	9.63 (9.21)	1.6
13j	COPh	Cl	207 (dec.) (IPA)	61	C ₂₃ H ₂₄ ClN ₃ O ₃ ·tartrate·H ₂ O	54.78 (54.99)	5.11 (5.07)	7.10 (7.21)	12
Azasetron									0.54

Table 3. 5-HT₃ Receptor Binding Affinity of Compounds **17a–f**


Compd. No.	R ²	R ³	mp (°C) (Recryst. solvent)	Yield (%)	Formula	Analysis (%) Calcd (Found)			[³ H]Granisetron binding K_i (nM)
						C	H	N	
17a	CH ₃	H	178–180 (EtOH–acetone)	43	C ₁₇ H ₂₃ N ₃ O ₂ ·fumarate·1/4H ₂ O	59.78 (59.79)	6.57 (6.75)	9.96 (10.18)	0.56
17b	CH ₃	F	201 (dec.) (EtOH–acetone)	75	C ₁₇ H ₂₂ FN ₃ O ₂ ·fumarate·1/4H ₂ O	57.33 (57.39)	6.07 (6.05)	9.55 (9.41)	0.14
17c	CH ₃	Br	198 (dec.) (EtOH)	63	C ₁₇ H ₂₂ BrN ₃ O ₂ ·tartrate·1/4H ₂ O	47.34 (47.24)	5.10 (5.28)	7.89 (7.84)	0.15
17d	CH ₃	CH ₃	234 (dec.) (EtOH–acetone)	28	C ₁₈ H ₂₅ N ₃ O ₂ ·1/2fumarate·1/4H ₂ O	63.56 (63.40)	7.33 (7.40)	11.12 (11.11)	4.1
17e	CH ₃	NO ₂	224–226 (AcOEt)	63	C ₁₇ H ₂₂ N ₄ O ₄	58.95 (58.67)	6.40 (6.34)	16.17 (16.02)	15
17f	CH ₃	NH ₂	148–149 (EtOH–acetone)	73	C ₁₇ H ₂₄ N ₄ O ₂ ·3maleate·1/4H ₂ O	52.06 (52.00)	5.50 (5.45)	8.37 (8.27)	4.2

(**13d**) normal-butyl (**13e**) > benzyl (**13g**) > isopropyl (**13f**) > phenethyl (**13h**). This result indicates that there is some steric hindrance at position 4 of the 1,4-benzoxazine ring in relation to the 5-HT₃ receptor. The 4-acetyl analog **13i** ($K_i = 1.6$ nM) also had a lower affinity relative to **13b**. The 4-benzoyl analog **13j** ($K_i = 12$ nM) was 7.5 times less potent

than **13i**. These results suggest that the presence of the basic nitrogen atom at position 4 of the benzoxazine ring reflects an interaction with the 5-HT₃ receptor, such as hydrogen bonding.

We subsequently attempted to optimize the substituent at the position 6 (Table 3). A decrease in affinity for the

Table 4. 5-HT₃ Receptor Antagonistic Activities of Compound **13b** and Its Enantiomers

Compd. No.	mp (°C) (Recryst. solvent)	Yield (%)	Formula	Analysis (%) Calcd (Found)			^[3H] Granisetron binding K_i (nM)	BJ reflex ^{a)} ED ₅₀ (μg/kg i.v.)
				C	H	N		
13b							0.072	0.28 (0.21—0.34)
(S)- 13b	185—186 ^{b)} (AcOEt)	74	C ₁₇ H ₂₂ ClN ₃ O ₂	60.80 (60.76)	6.60 (6.61)	12.51 (12.51)	0.051	0.089 (0.089—0.10)
(R)- 13b	185—186 ^{c)} (AcOEt)	73	C ₁₇ H ₂₂ ClN ₃ O ₂	60.80 (60.71)	6.60 (6.60)	12.51 (12.48)	0.54	0.73 (0.57—0.86)
Granisetron							0.41	0.74 (0.47—1.07)
Zacopride							0.18	0.5 (0.40—0.63)
Azasetron							0.54	1.3 (0.9—2.0)

a) Serotonin was administered at a dose of 10 μg/kg i.v. 5 min posttreatment with a drug at the specified dose. Values in parentheses indicate the 95% confidence limits. b) $[\alpha]_D^{25} = -16.3$ (c = 1.0, EtOH). c) $[\alpha]_D^{25} = +16.7$ (c = 1.0, EtOH).

Table 5. ¹H-NMR Spectral Data for **7a—c**, **13a—j**, and **17a—f** (100 MHz)

7a	1.42—1.84 (4H, m), 1.95—2.12 (1H, m), 2.33—2.73 (2H, m), 2.84 (6H, s), 2.75—3.05 (3H, m), 3.28—3.60 (1H, m), 3.84 (3H, s), 4.00—4.31 (1H, m), 6.93 (1H, d, $J=3$), 7.62 (1H, d, $J=3$), 8.09—8.36 (1H, brs)
(CDCl ₃)	
7b	1.40—1.83 (4H, m), 1.91—2.08 (1H, m), 2.40—2.71 (1H, m), 2.72—3.01 (4H, m), 3.25—3.58 (1H, m), 3.79 (3H, s), 3.81—4.03 (2H, brs), 4.04—4.26 (1H, m), 6.81 (1H, d, $J=3$), 7.33 (1H, d, $J=3$), 7.70—8.00 (1H, brs)
(CDCl ₃)	
7c	1.53—2.04 (4H, m), 1.80 (3H, s), 2.05—2.27 (1H, m), 2.83—3.43 (5H, m), 3.11 (3H, s), 3.44—3.82 (1H, m), 3.82 (3H, s), 4.21—4.48 (1H, m), 7.51 (1H, d, $J=3$), 7.67 (1H, d, $J=3$), 8.65—8.99 (1H, brs)
(DMSO- <i>d</i> ₆)	
13a	1.50—2.04 (4H, m), 2.06—2.26 (1H, m), 2.83—3.28 (5H, m), 3.32 (2H, t, $J=4$), 3.36—3.82 (1H, m), 4.01—4.22 (1H, m), 4.20 (2H, t, $J=4$), 4.28—4.52 (1H, brs), 6.69 (2H, s), 8.36—8.43 (1H, brs), 10.60—10.88 (1H, brs)
(DMSO- <i>d</i> ₆)	
13b	1.66—1.91 (4H, m), 1.94—2.06 (1H, m), 2.87 (3H, s), 3.03—3.25 (5H, m), 3.29 (2H, t, $J=4$), 3.47—3.62 (1H, m), 4.18—4.28 (1H, m), 4.29 (2H, t, $J=4$), 6.72 (1H, d, $J=3$), 6.75 (1H, d, $J=3$), 8.42—8.42 (1H, brs), 10.53—10.64 (1H, brs)
(DMSO- <i>d</i> ₆)	
13c	1.08 (3H, t, $J=5$), 1.22—1.91 (4H, m), 1.94—2.18 (1H, m), 2.63—3.18 (5H, m), 3.19—3.47 (1H, m), 3.33 (2H, t, $J=4$), 3.43 (2H, q, $J=5$), 3.97—4.31 (1H, m), 4.24 (2H, t, $J=4$), 7.22 (1H, d, $J=3$), 6.48 (2H, s, fumarate), 6.67 (1H, d, $J=3$), 6.76 (1H, d, $J=3$), 8.22—8.40 (1H, brs)
(DMSO- <i>d</i> ₆)	
13d	0.90 (3H, t, $J=7$), 1.26—1.92 (6H, m), 1.94—2.15 (1H, m), 2.60—3.12 (5H, m), 3.13—3.58 (3H, m), 3.36 (2H, t, $J=4$), 3.92—4.22 (1H, m), 4.22 (2H, t, $J=4$), 6.45 (2H, s, fumarate), 6.63 (1H, d, $J=3$), 6.72 (1H, d, $J=3$), 8.16—8.40 (1H, brs)
(DMSO- <i>d</i> ₆)	
13e	0.92 (3H, t, $J=5$), 1.11—1.62 (4H, m), 1.63—1.91 (4H, m), 1.93—2.14 (1H, m), 2.76—3.13 (5H, m), 3.16—3.59 (3H, m), 3.37 (2H, t, $J=4$), 4.21 (2H, t, $J=4$), 6.48 (2H, s, fumarate), 6.65 (1H, d, $J=3$), 6.37 (1H, d, $J=3$), 8.20—8.39 (1H, brs)
(DMSO- <i>d</i> ₆)	
13f	0.89 (6H, d, $J=7$), 1.20—1.96 (4H, m), 1.96—2.20 (2H, m), 2.61—3.19 (7H, m), 3.21—3.52 (1H, m), 3.40 (2H, t, $J=4$), 3.92—4.31 (1H, m), 4.21 (2H, t, $J=4$), 6.49 (2H, s, fumarate), 6.65 (1H, d, $J=3$), 6.63 (1H, d, $J=3$), 6.68 (1H, d, $J=3$), 8.20—8.42 (1H, brs)
(DMSO- <i>d</i> ₆)	
13g	1.18—1.92 (4H, m), 1.97—2.12 (1H, m), 2.70—3.21 (5H, m), 3.23—3.50 (1H, m), 3.48 (2H, t, $J=4$), 3.98—4.24 (1H, m), 4.30 (2H, t, $J=4$), 4.58 (2H, s), 6.48 (1H, s), 6.70 (1H, s), 7.12—7.44 (5H, m), 8.21—8.43 (1H, brs)
(DMSO- <i>d</i> ₆)	
13h	1.44—1.98 (4H, m), 1.98—2.29 (1H, m), 2.81 (2H, t, $J=5$), 2.88—3.31 (7H, m), 3.30 (2H, t, $J=4$), 3.54 (2H, t, $J=5$), 4.15 (2H, t, $J=4$), 6.52 (2H, s, fumarate), 6.66 (1H, d, $J=2$), 6.77 (1H, d, $J=2$), 7.10—7.34 (5H, m), 8.22—8.44 (1H, brs)
(DMSO- <i>d</i> ₆)	
13i	1.48—2.07 (4H, m), 2.10—2.24 (1H, m), 2.28 (3H, s), 2.80—3.39 (5H, m), 3.62—3.94 (1H, m), 3.88 (2H, t, $J=4$), 4.08—4.32 (1H, m), 4.36 (2H, t, $J=4$), 7.25 (1H, d, $J=3$), 7.94—8.12 (1H, m), 8.50—8.63 (1H, brs), 10.48—10.87 (1H, brs)
(DMSO- <i>d</i> ₆)	
13j	1.41—1.98 (4H, m), 1.99—2.20 (1H, m), 2.73—3.25 (5H, m), 3.22—3.63 (1H, m), 3.88 (2H, t, $J=4$), 3.98 (2H, s, tartrate), 3.98—4.33 (1H, m), 4.38 (2H, t, $J=4$), 7.22 (1H, d, $J=3$), 7.45 (1H, d, $J=3$), 7.33—7.63 (5H, m), 8.36—8.52 (1H, brs)
(DMSO- <i>d</i> ₆)	
17a	1.41—1.97 (4H, m), 1.98—2.21 (1H, m), 2.84 (3H, s), 2.88—3.18 (5H, m), 3.26 (2H, t, $J=5$), 3.22—3.62 (1H, m), 3.98—4.28 (1H, m), 4.31 (2H, t, $J=5$), 6.44 (2H, s, fumarate), 6.68—6.90 (3H, m), 8.10—8.27 (1H, brs)
(DMSO- <i>d</i> ₆)	
17b	1.41—1.92 (4H, m), 1.98—2.16 (1H, m), 2.87 (3H, s), 2.90—3.18 (5H, m), 3.31 (2H, t, $J=4$), 3.32—3.61 (1H, m), 3.96—4.33 (1H, m), 4.28 (2H, t, $J=4$), 6.21 (1H, dd, $J=3, 8$), 6.47 (2H, s, fumarate), 6.62 (1H, dd, $J=3, 8$), 8.21—8.40 (1H, brs)
(DMSO- <i>d</i> ₆)	
17c	1.42—1.96 (4H, m), 1.98—2.20 (1H, m), 2.88 (3H, s), 2.96—3.22 (5H, m), 3.30 (2H, t, $J=4$), 3.32—3.69 (1H, m), 3.98 (2H, s, tartrate), 4.00—4.27 (1H, m), 4.29 (2H, t, $J=4$), 6.85 (2H, s), 8.20—8.44 (1H, brs)
(DMSO- <i>d</i> ₆)	
17d	1.32—1.88 (4H, m), 1.89—2.04 (1H, m), 2.20 (3H, s), 2.56—3.05 (5H, m), 2.85 (3H, s), 3.25 (2H, t, $J=4$), 3.22—3.50 (1H, m), 3.88—4.19 (1H, m), 4.30 (2H, t, $J=4$), 6.44 (1H, s, fumarate), 6.59 (1H, d, $J=2$), 6.68 (1H, d, $J=3$), 8.04—8.24 (1H, brs)
(DMSO- <i>d</i> ₆)	
17e	1.15—1.72 (4H, m), 1.78—1.96 (1H, m), 2.52—2.86 (5H, m), 2.96 (3H, s), 2.98—3.28 (1H, m), 3.37 (2H, t, $J=4$), 3.71—4.03 (1H, m), 4.43 (2H, t, $J=4$), 7.41 (1H, d, $J=3$), 7.63 (1H, d, $J=3$), 8.08—8.32 (1H, brs)
(DMSO- <i>d</i> ₆)	
17f	1.58—2.03 (4H, m), 2.06—2.24 (1H, m), 2.81 (3H, s), 2.92—3.38 (5H, m), 3.22 (2H, t, $J=4$), 3.41—3.80 (1H, m), 4.09—4.24 (1H, m), 4.25 (2H, t, $J=4$), 6.00—6.20 (2H, brs), 6.12 (6H, s, maleate), 6.31 (1H, d, $J=2$), 6.43 (1H, d, $J=2$), 8.18—8.38 (1H, brs)
(DMSO- <i>d</i> ₆)	

5-HT₃ receptor was observed when the Cl atom at position 6 was removed (compound **17a**). The 6-fluoro and 6-bromo compounds **17b** and **17c** showed slightly less activity than the 6-chloro compound **13b**. Replacement of the chloro group at position 6 by a methyl group resulted in reduced activity (**17d**, $K_i = 4.1$ nM). The 6-nitro compound **17e** ($K_i = 15$ nM) was 20 times less potent than **13b**. The introduction of an amino group at position 6 of benzoxazine ring did not contribute to the enhancement of the pharmacological activity.

Compound **13b** is separable to two enantiomers, because it has a chiral center on its quinuclidine ring. We synthesized the (*S*)- and (*R*)-enantiomer of **13b** to compare their pharmacological activity. We found that (*S*)-**13b** is one order of magnitude more potent than its counterpart as shown in Table 4. Compound (*S*)-**13b** also showed potent antagonistic activity against the von Bezold-Jarisch reflex ($ED_{50} = 0.089$ μ g/kg i.v.) in rats.

In conclusion, we found that the 4-amino substituent on the aromatic moiety of zacopride was replaceable by a 3-dimethylamino substituent with retention of potent 5-HT₃ receptor antagonistic activity. On the basis of this result, we designed 3,4-dihydro-2*H*-1,4-benzoxazine-8-carboxamide derivatives and found that (*S*)-*N*-(1-azabicyclo[2.2.2]oct-3-yl)-6-chloro-3,4-dihydro-4-methyl-2*H*-1,4-benzoxazine-8-carboxamide (*S*)-**13b** showed high affinity for 5-HT₃ receptors and potent antagonistic activity against the von Bezold-Jarisch reflex. The pharmacological superiority of **7a** and (*S*)-**13b** over their parent compounds suggests that the basic nitrogen atom at position 3 of the benzamides contributes to the host-guest binding on the 5-HT₃ receptor.

Experimental

Melting points were determined in open capillaries and are uncorrected. Proton nuclear magnetic resonance (¹H-NMR) spectra were recorded on a JEOL PS-100 spectrometer and the chemical shifts were expressed in ppm downfield from tetramethylsilane as an internal standard. Low-resolution mass spectra (MS) were obtained with a JMS-01SG spectrometer. Optical rotations were obtained on a JASCO DIP-181 digital polarimeter. Elemental analysis and measurement of these spectra were performed in our laboratory. Granisetron, and zacopride were prepared by the reported methods^{12a,b} in our laboratory.

Ethyl 5-Chloro-3-dimethylamino-2-methoxybenzoate (2) Methyl iodide (5 ml, 80 mmol) was added to a mixture of **1** (4.6 g, 23 mmol), dimethylformamide (DMF, 70 ml), and K₂CO₃ (13 g, 92 mmol) with stirring at room temperature, and the mixture was heated at 70–75 °C for an additional 3 h. The mixture was poured into a two-layer mixture of ice-water (50 ml) and ethyl acetate (AcOEt, 50 ml). The organic layer was separated, washed successively with water and saturated brine, and dried over MgSO₄. The organic layer was evaporated *in vacuo* to afford an oil, which was chromatographed on silica gel to give **2** as an oil (4.2 g, 71%). ¹H-NMR (CDCl₃) δ : 1.39 (3H, t, $J = 6$ Hz), 2.84 (6H, s), 2.80 (3H, s), 4.35 (2H, q, $J = 6$ Hz), 6.92 (1H, d, $J = 2$ Hz), 7.17 (1H, d, $J = 2$ Hz). MS m/z : 257 (M^+).

5-Chloro-3-dimethylamino-2-methoxybenzoic Acid (3a) A mixture of **2** (3.2 g, 12 mmol), sodium hydroxide (1.0 g, 25 mmol), water (30 ml), and methanol (MeOH, 10 ml) was stirred at room temperature overnight. The reaction mixture was then acidified to pH 5–6 with concentrated hydrochloric acid, and extracted with chloroform (CHCl₃, 2 $\times$ 100 ml). The organic extracts were dried over MgSO₄ and evaporated under reduced pressure to afford a white solid. The resulting solid was recrystallized from EtOH to give **3a** (2.0 g, 70%) as colorless crystals, mp 63–64 °C. ¹H-NMR (CDCl₃) δ : 2.84 (6H, s), 3.99 (3H, s), 7.03 (1H, d, $J = 3$ Hz), 7.63 (1H, d, $J = 3$ Hz). Anal. Calcd for C₁₀H₁₂ClNO₃: C, 52.30; H, 5.27; N, 6.10. Found: C, 52.01; H, 5.25; N, 5.99. MS m/z : 229

(M^+).

***N*-(3-Ethoxycarbonyl-5-chloro-2-hydroxyphenyl)acetamide (4)** Acetyl chloride (7.2 g, 92 mmol) was added dropwise to a solution of **1** (10 g, 46 mmol), CHCl₃ (500 ml), and the mixture was aqueous saturated NaHCO₃ (500 ml) with vigorous stirring at 0–10 °C, and stirred for an additional 2 h. The separated organic layer was washed with water, and dried over MgSO₄. The organic layer was evaporated *in vacuo* to afford a solid, which was recrystallized from EtOH–diisopropyl ether (IPE) to give **4** (11 g, 93%) as colorless crystals, mp 121–123 °C. ¹H-NMR (CDCl₃) δ : 1.40 (3H, t, $J = 7$ Hz), 2.23 (3H, s), 4.43 (2H, q, $J = 7$ Hz), 7.51 (1H, d, $J = 2$ Hz), 7.73–7.88 (1H, br s), 8.76 (1H, d, $J = 2$ Hz), 11.32 (1H, s). Anal. Calcd for C₁₁H₁₂ClNO₄: C, 51.27; H, 4.69; N, 5.44. Found: C, 51.12; H, 4.66; N, 5.45. MS m/z : 257 (M^+).

***N*-(3-Ethoxycarbonyl-5-chloro-2-methoxyphenyl)acetamide (5)** Methyl iodide (1.5 ml, 23 mmol) was added to a mixture of **4** (5.0 g, 19 mmol), acetone (70 ml), and K₂CO₃ (3.2 g, 23 mmol) with stirring at room temperature for an additional 3 h. The mixture was poured into a two-layer mixture of ice-water (50 ml) and AcOEt (50 ml). The organic layer was separated, washed successively with water and saturated brine, and dried over MgSO₄. The organic layer was evaporated *in vacuo* to afford a solid, which was recrystallized from EtOH–IPE to give **5** (4.4 g, 86%) as colorless crystals, mp 57–59 °C. ¹H-NMR (CDCl₃) δ : 1.41 (3H, t, $J = 7$ Hz), 2.23 (3H, s), 3.87 (3H, s), 4.38 (2H, q, $J = 7$ Hz), 7.51 (1H, d, $J = 2$ Hz), 7.78–7.93 (1H, br s), 8.64 (1H, d, $J = 2$ Hz). Anal. Calcd for C₁₂H₁₄ClNO₄: C, 53.05; H, 5.19; N, 5.16. Found: C, 52.94; H, 5.05; N, 5.32. MS m/z : 271 (M^+).

3-Amino-5-chloro-2-methoxybenzoic Acid Hydrochloride (3b) A mixture of **5** (4.4 g, 16 mmol), sodium hydroxide (2.1 g, 48 mmol), water (30 ml), and MeOH (10 ml) was refluxed for 5 h. Additional water was added, and the reaction mixture was extracted with AcOEt. The reaction mixture was then acidified to pH 5–6 with concentrated hydrochloric acid, and extracted with CHCl₃ (2 $\times$ 100 ml). The organic extracts were dried over MgSO₄ and evaporated under reduced pressure to afford a white solid. The resulting solid was recrystallized from EtOH to give **3b** (2.4 g, 75%) as colorless crystals, mp 182–184 °C. ¹H-NMR (DMSO-*d*₆) δ : 3.74 (3H, s), 7.08 (1H, d, $J = 2$ Hz), 7.18 (1H, d, $J = 2$ Hz), 7.84–8.66 (2H, br s). Anal. Calcd for C₈H₈ClNO₃ · HCl · 1/2H₂O: C, 38.85; H, 3.94; N, 5.61. Found: C, 38.89; H, 4.08; N, 5.67. MS m/z : 201 (M^+).

***N*-(3-Ethoxycarbonyl-5-chloro-2-methoxyphenyl)-*N*-methylacetamide (6)** Methyl iodide (5 ml, 80 mmol) was added to a mixture of **5** (5.0 g, 18 mmol), DMF (70 ml), and potassium *tert*-butoxide (2.2 g, 12 mmol) with stirring at room temperature, and the mixture was heated at 70–75 °C for an additional 3 h. The mixture was poured into a two-layer mixture of ice-water (50 ml) and AcOEt (50 ml). The organic layer was separated, washed successively with water and saturated brine, and dried over MgSO₄. The organic layer was evaporated *in vacuo* to afford an oil, which was chromatographed on silica gel to give **6** as an oil (3.1 g, 61%). ¹H-NMR (CDCl₃) δ : 1.42 (3H, t, $J = 6$ Hz), 1.91 (3H, s), 3.24 (3H, s), 3.82 (3H, s), 4.41 (2H, q, $J = 6$ Hz), 7.35 (1H, d, $J = 2$ Hz), 7.75 (1H, d, $J = 2$ Hz). MS m/z : 285 (M^+).

5-Chloro-2-methoxy-3-(*N*-methylacetylaminobenzoic Acid (3c) A mixture of **6** (3.1 g, 11 mmol), sodium hydroxide (0.7 g, 17 mmol), water (20 ml), and MeOH (7 ml) was stirred at room temperature overnight. The reaction mixture was then acidified with concentrated hydrochloric acid, and the precipitates were collected and washed with cold water. The resulting solid was recrystallized from EtOH to give **3c** (2.6 g, 91%) as colorless crystals, mp 130–132 °C. ¹H-NMR (CDCl₃) δ : 1.99 (3H, s), 3.31 (3H, s), 3.92 (3H, s), 7.43 (1H, d, $J = 2$ Hz), 7.99 (1H, d, $J = 2$ Hz). Anal. Calcd for C₁₁H₁₂ClNO₄: C, 51.27; H, 4.69; N, 5.44. Found: C, 50.99; H, 4.72; N, 5.21. MS m/z : 257 (M^+).

General Procedure for the Preparation of 3-Substituted *N*-(1-Azabicyclo[2.2.2]oct-3-yl)-5-chloro-2-methoxybenzamides (7a–c) Physical and spectral data for compounds **7a–c** are listed in Tables 1 and 5. Isobutyl chloroformate (1.3 ml, 10 mmol) was added to a mixture of the carboxylic acid **3a**, **3b** or **3c** (10 mmol), NEt₃ (1.4 ml, 10 mmol), and AcOEt (30 ml) at –10 °C. The mixture was stirred below –5 °C for 30 min, and a solution of 3-amino-1-aza-bicyclo[2.2.2]octane (1.3 g, 10 mmol) in AcOEt (5 ml) was added with stirring at –10 °C. Stirring was continued at –10 °C for 30 min and then at room temperature for 1 h, water was added and the resulting mixture was extracted with AcOEt. The extract was washed with water, dried over MgSO₄ and evaporated to dryness. Compounds **7a** and **7b** were recrystallized from AcOEt and **7c** was converted to the oxalate in the usual manner.

Ethyl 4-Acetyl-6-chloro-3,4-dihydro-2*H*-1,4-benzoxazine-8-carboxyl-

ate (8) Dibromoethane (17 ml, 0.14 mol) was added to a mixture of **4** (31 g, 0.12 mol), DMF (700 ml), and K_2CO_3 (41 g, 0.30 mol) with stirring at room temperature, and the mixture was heated at 70–75 °C for an additional 3 h. The mixture was poured into a two-layer mixture of ice-water (500 ml) and AcOEt (500 ml). The organic layer was separated, washed successively with water and saturated brine, and dried over $MgSO_4$. The organic layer was evaporated *in vacuo* to afford a solid, which was recrystallized from EtOH–IPE to give **8** (30 g, 88%) as colorless crystals, mp 89–90 °C. 1H -NMR ($CDCl_3$) δ : 1.38 (3H, t, J = 7 Hz), 2.30 (3H, s), 3.91 (2H, t, J = 4 Hz), 4.34 (2H, q, J = 7 Hz), 4.40 (3H, t, J = 4 Hz), 7.54 (2H, d, J = 2 Hz). *Anal.* Calcd for $C_{13}H_{14}ClNO_4$: C, 55.04; H, 4.97; N, 4.94. Found: C, 54.92; H, 4.96; N, 5.00. MS m/z : 283 (M^+).

6-Chloro-3,4-dihydro-2H-1,4-benzoxazine-8-carboxylic Acid (9) A mixture of **8** (30 g, 0.11 mol), sodium hydroxide (21 g, 0.55 mol), water (1000 ml), and MeOH (300 ml) was refluxed for 3 h. Additional water was added, and the reaction mixture was extracted with ethyl acetate. The reaction mixture was then acidified to pH 5–6 with concentrated hydrochloric acid, and extracted with chloroform (2 $\times$ 100 ml). The organic extracts were dried over $MgSO_4$ and evaporated at reduced pressure to afford a white solid. The resulting solid was recrystallized from EtOH to give **9** (21 g, 93%) as colorless crystals, mp 62–63 °C. 1H -NMR ($DMSO-d_6$) δ : 3.51 (2H, t, J = 3 Hz), 4.16 (3H, t, J = 3 Hz), 6.10–6.49 (1H, br s), 6.69 (1H, d, J = 2 Hz), 6.75 (1H, d, J = 2 Hz). *Anal.* Calcd for $C_9H_8ClNO_3$: C, 50.60; H, 3.77; N, 6.56. Found: C, 50.42; H, 3.81; N, 6.60. MS m/z : 213 (M^+).

Methyl 6-Chloro-3,4-dihydro-2H-1,4-benzoxazine-8-carboxylate (10) Concentrated sulfuric acid (50 ml, 0.20 mol) was added dropwise to a mixture of **9** (32 g, 0.15 mol) and MeOH (500 ml) with stirring at 10 °C, and the mixture was refluxed for an additional 10 h. After evaporation of the solvent, the residue was poured into a two-layer mixture of ice-water (500 ml) and AcOEt (500 ml). The organic layer was separated, washed successively with water and saturated brine, and dried over $MgSO_4$. The organic layer was evaporated *in vacuo* to afford a solid, which was recrystallized from hexane–IPE to give **10** (32 g, 95%) as colorless crystals, mp 62–63 °C. 1H -NMR ($DMSO-d_6$) δ : 3.31 (2H, t, J = 4 Hz), 3.75 (3H, s), 4.14 (2H, t, J = 4 Hz), 6.12–6.48 (1H, br s), 7.70 (1H, d, J = 2 Hz), 7.76 (1H, d, J = 2 Hz). *Anal.* Calcd for $C_{10}H_{10}ClNO_3$: C, 52.76; H, 4.43; N, 6.15. Found: C, 52.76; H, 4.46; N, 6.10. MS m/z : 227 (M^+).

6-Chloro-3,4-dihydro-4-methyl-2H-1,4-benzoxazine-8-carboxylic Acid (12b) Methyl iodide (5 ml, 80 mmol) was added to a mixture of **10** (9.1 g, 40 mmol), DMF (100 ml), and K_2CO_3 (13 g, 92 mmol) with stirring at room temperature, and the mixture was heated at 70–75 °C for an additional 3 h. The mixture was poured into a two-layer mixture of ice-water (100 ml) and AcOEt (100 ml). The organic layer was separated, washed successively with water and saturated brine, and dried over $MgSO_4$. The organic layer was evaporated *in vacuo* to afford **11b** (7.7 g, 80%) as an oil. A mixture of **11b**, sodium hydroxide (2.6 g, 60 mmol), water (100 ml), and MeOH (45 ml) was heated at 50–55 °C for 3 h. The reaction mixture was then acidified to pH 5–6 with concentrated hydrochloric acid, and extracted with $CHCl_3$ (2 $\times$ 100 ml). The organic extracts were dried over $MgSO_4$ and evaporated under reduced pressure to afford a white solid. The resulting solid was recrystallized from EtOH to give **12b** (6.9 g, 76%) as colorless crystals, mp 202–203 °C. 1H -NMR ($DMSO-d_6$) δ : 2.87 (3H, s), 3.29 (2H, t, J = 4 Hz), 4.25 (2H, t, J = 4 Hz), 6.75 (1H, d, J = 2 Hz), 6.82 (1H, d, J = 2 Hz). *Anal.* Calcd for $C_{10}H_{10}ClNO_3$: C, 52.76; H, 4.43; N, 6.15. Found: C, 52.72; H, 4.47; N, 6.12. MS m/z : 227 (M^+).

6-Chloro-3,4-dihydro-4-ethyl-2H-1,4-benzoxazine-8-carboxylic Acid (12c) Acetaldehyde (1.7 ml, 31 mmol) was added to a mixture of **10** (7.0 g, 31 mmol), MeOH (100 ml), 15% EtOH–HCl (5 ml), and sodium cyanoborohydride (1.9 g, 31 mmol) with stirring below 5 °C, and the mixture was stirred at room temperature for an additional 8 h. After evaporation of the solvent, the residue was poured into a two-layer mixture of aqueous K_2CO_3 (100 ml) and AcOEt (100 ml). The organic layer was separated, washed successively with water and saturated brine, and dried over $MgSO_4$. The organic layer was evaporated *in vacuo* to afford **11c** as an oil, which was used in the next reaction without further purification. A mixture of **11c**, sodium hydroxide (2.6 g, 60 mmol), water (100 ml), and MeOH (45 ml) was heated at 50–55 °C for 3 h. The reaction mixture was then acidified to pH 5–6 with concentrated hydrochloric acid, and extracted with $CHCl_3$ (2 $\times$ 100 ml). The organic extracts were dried over $MgSO_4$ and evaporated under reduced pressure

to afford a white solid. The resulting solid was recrystallized from EtOH–IPE to give **12c** (3.8 g, 51%) as colorless crystals, mp 76–77 °C. 1H -NMR ($DMSO-d_6$) δ : 1.30 (3H, t, J = 7 Hz), 3.82 (2H, t, J = 4 Hz), 4.28 (2H, q, J = 7 Hz), 4.29 (2H, t, J = 4 Hz), 7.40 (1H, d, J = 2 Hz), 7.87 (1H, d, J = 2 Hz). *Anal.* Calcd for $C_{11}H_{12}ClNO_3$: C, 54.67; H, 5.00; N, 5.80. Found: C, 54.43; H, 5.05; N, 5.92. MS m/z : 241 (M^+).

Compounds **12d–h** were prepared by the same procedure as described for **12c**.

6-Chloro-3,4-dihydro-4-propyl-2H-1,4-benzoxazine-8-carboxylic Acid (12d) Yield 45%, mp 136–137 °C (EtOH–IPE). 1H -NMR ($DMSO-d_6$) δ : 0.99 (3H, t, J = 7 Hz), 1.42–1.84 (2H, m), 3.23 (2H, t, J = 7 Hz), 3.47 (2H, t, J = 3 Hz), 4.43 (2H, t, J = 3 Hz), 6.71 (1H, d, J = 2 Hz), 6.33 (1H, d, J = 2 Hz). *Anal.* Calcd for $C_{12}H_{14}ClNO_3$: C, 56.37; H, 5.52; N, 5.48. Found: C, 56.34; H, 5.50; N, 5.47. MS m/z : 255 (M^+).

4-Butyl-6-chloro-3,4-dihydro-2H-1,4-benzoxazine-8-carboxylic Acid (12e) Yield 32%, mp 116–118 °C (EtOH–IPE). 1H -NMR ($DMSO-d_6$) δ : 0.92 (3H, t, J = 7 Hz), 1.06–1.71 (4H, m), 3.01–3.64 (4H, m), 4.19 (2H, t, J = 4 Hz), 6.72 (1H, d, J = 2 Hz), 6.78 (1H, d, J = 2 Hz). *Anal.* Calcd for $C_{13}H_{16}ClNO_3$: C, 57.89; H, 5.98; N, 5.19. Found: C, 57.69; H, 6.02; N, 4.98. MS m/z : 269 (M^+).

6-Chloro-3,4-dihydro-4-isobutyl-2H-1,4-benzoxazine-8-carboxylic Acid (12f) Yield 49%, mp 124–125 °C (EtOH–IPE). 1H -NMR ($DMSO-d_6$) δ : 0.91 (6H, t, J = 6 Hz), 1.70–2.23 (1H, m), 3.08 (2H, d, J = 7 Hz), 3.38 (2H, t, J = 4 Hz), 4.17 (2H, t, J = 4 Hz), 6.67–6.83 (2H, m). *Anal.* Calcd for $C_{13}H_{16}ClNO_3$: C, 57.89; H, 5.98; N, 5.19. Found: C, 58.11; H, 6.09; N, 5.13. MS m/z : 269 (M^+).

4-Benzyl-6-chloro-3,4-dihydro-2H-1,4-benzoxazine-8-carboxylic Acid (12g) Yield 61%, mp 115–116 °C (EtOH–IPE). 1H -NMR ($DMSO-d_6$) δ : 3.46 (2H, t, J = 4 Hz), 4.25 (2H, t, J = 4 Hz), 4.55 (2H, s), 6.69 (1H, d, J = 2 Hz), 6.76 (1H, d, J = 2 Hz), 7.08–7.45 (5H, m). *Anal.* Calcd for $C_{16}H_{14}ClNO_3$: C, 63.27; H, 4.65; N, 4.61. Found: C, 62.95; H, 4.76; N, 4.59. MS m/z : 303 (M^+).

6-Chloro-3,4-dihydro-4-(2-phenylethyl)-2H-1,4-benzoxazine-8-carboxylic Acid (12h) Yield 40%, mp 128–129 °C (EtOH–IPE). 1H -NMR ($DMSO-d_6$) δ : 2.82 (2H, t, J = 4 Hz), 3.28 (2H, t, J = 4 Hz), 3.54 (2H, t, J = 4 Hz), 4.11 (2H, t, J = 4 Hz), 6.76 (1H, d, J = 2 Hz), 6.81 (1H, d, J = 2 Hz), 7.19–7.38 (5H, m). *Anal.* Calcd for $C_{17}H_{16}ClNO_3$: C, 64.26; H, 5.08; N, 4.41. Found: C, 64.06; H, 5.04; N, 4.37. MS m/z : 317 (M^+).

4-Acetyl-6-chloro-3,4-dihydro-2H-1,4-benzoxazine-8-carboxylic Acid (12i) Acetyl chloride (2.4 ml, 34 mmol) was added to a mixture of **10** (7.0 g, 31 mmol), dichloromethane (100 ml), and Et_3N (4.8 ml, 34 mmol) with stirring below 5 °C, and the mixture was stirred at room temperature for an additional 3 h. The mixture was poured into a two-layer mixture of water (100 ml) and AcOEt (100 ml). The organic layer was separated, washed successively with water and saturated brine, and dried over $MgSO_4$. The organic layer was evaporated *in vacuo* to afford **11i** as an oil, which was used in the next reaction without further purification. A mixture of **11i**, sodium hydroxide (2.6 g, 60 mmol), water (100 ml), and MeOH (45 ml) was stirred at room temperature overnight. The reaction mixture was then acidified to pH 5–6 with concentrated hydrochloric acid, and extracted with $CHCl_3$ (2 $\times$ 100 ml). The organic extracts were dried over $MgSO_4$ and evaporated under reduced pressure to afford a white solid. The resulting solid was recrystallized from EtOH–IPE to give **12i** (5.1 g, 64%) as colorless crystals, mp 76–77 °C. 1H -NMR ($DMSO-d_6$) δ : 2.27 (3H, s), 3.87 (2H, t, J = 4 Hz), 4.35 (2H, t, J = 4 Hz), 7.37 (1H, d, J = 2 Hz), 7.82–8.19 (1H, m). *Anal.* Calcd for $C_{11}H_{10}ClNO_4$: C, 51.68; H, 3.94; N, 5.48. Found: C, 51.70; H, 4.06; N, 5.53. MS m/z : 255 (M^+).

Compound **12j** was prepared by the same procedure as described for **12i**.

4-Benzoyl-6-chloro-3,4-dihydro-2H-1,4-benzoxazine-8-carboxylic Acid (12j) Yield 63%, mp 175–176 °C (EtOH–IPE). 1H -NMR ($DMSO-d_6$) δ : 3.86 (2H, t, J = 4 Hz), 4.37 (2H, t, J = 4 Hz), 7.37 (1H, d, J = 2 Hz), 7.56 (1H, d, J = 2 Hz), 7.41–7.68 (5H, m). *Anal.* Calcd for $C_{16}H_{12}ClNO_4$: C, 60.48; H, 3.81; N, 4.41. Found: C, 60.18; H, 3.76; N, 4.41. MS m/z : 317 (M^+).

Ethyl 3,4-Dihydro-2H-1,4-benzoxazine-8-carboxylate (15a) Key intermediates **15a–d** were prepared from the amides **14a–d**⁷⁾ by Merkel's method⁸⁾ Boron trifluoride diethyl etherate (10.3 ml, 84 mmol) was added dropwise to a mixture of **14a** (8.8 g, 40 mmol) and tetrahydrofuran (THF, 100 ml) with stirring below 5 °C. The mixture was stirred at 5 °C for another 20 min, then sodium borohydride (3.2 g, 84 mmol) was added dropwise to the reaction mixture below 5 °C. The whole was kept for 1 h at 5 °C, then AcOEt (50 ml) was added dropwise, followed by

addition of 1 N HCl (50 ml). The aqueous layer was added basic with aqueous K_2CO_3 , and extracted with $CHCl_3$ (2×100 ml). The organic extracts were dried over $MgSO_4$ and evaporated under reduced pressure to afford an oil, which was chromatographed on silica gel to give **15a** as an oil (6.5 g, 79%). 1H -NMR ($CDCl_3$) δ : 1.36 (3H, t, $J=7$ Hz), 3.44 (2H, t, $J=4$ Hz), 3.75–3.98 (1H, brs), 4.32 (2H, q, $J=7$ Hz), 4.41 (2H, t, $J=4$ Hz), 6.71 (1H, d, $J=2$ Hz), 6.76 (1H, d, $J=8$ Hz), 7.14 (1H, dd, $J=2, 8$ Hz). MS m/z : 207 (M^+).

Compounds **15b–d** were prepared by the same procedure as described for **15a**.

Ethyl 3,4-Dihydro-6-fluoro-2H-1,4-benzoxazine-8-carboxylate (15b) Yield 83%, pale yellow oil. 1H -NMR ($CDCl_3$) δ : 1.36 (3H, t, $J=7$ Hz), 3.43 (2H, t, $J=4$ Hz), 3.95–4.18 (1H, brs), 4.28 (2H, t, $J=4$ Hz), 4.30 (2H, q, $J=7$ Hz), 6.42 (1H, dd, $J=3, 9$ Hz), 6.79 (1H, dd, $J=3, 9$ Hz). MS m/z : 207 (M^+).

Ethyl 6-Bromo-3,4-dihydro-2H-1,4-benzoxazine-8-carboxylate (15c) Yield 74%, pale yellow oil. 1H -NMR ($CDCl_3$) δ : 1.36 (3H, t, $J=7$ Hz), 3.42 (2H, t, $J=4$ Hz), 3.87–4.09 (1H, brs), 4.30 (2H, t, $J=4$ Hz), 4.31 (2H, q, $J=7$ Hz), 6.73 (1H, d, $J=2$ Hz), 7.02 (1H, d, $J=2$ Hz). MS m/z : 285 (M^+).

Ethyl 3,4-Dihydro-6-methyl-2H-1,4-benzoxazine-8-carboxylate (15d) Yield 81%, pale yellow oil. 1H -NMR ($CDCl_3$) δ : 1.47 (3H, t, $J=7$ Hz), 2.29 (3H, s), 3.50 (2H, t, $J=4$ Hz), 3.72–3.87 (1H, brs), 4.39 (2H, t, $J=4$ Hz), 4.43 (2H, q, $J=7$ Hz), 6.62 (1H, d, $J=2$ Hz), 7.05 (1H, d, $J=2$ Hz). MS m/z : 221 (M^+).

3,4-Dihydro-4-methyl-2H-1,4-benzoxazine-8-carboxylic Acid (16a) Methyl iodide (5 ml, 80 mmol) was added to a mixture of **15a** (7.2 g, 40 mmol), DMF (100 ml), and K_2CO_3 (13 g, 92 mmol) with stirring at room temperature, and the whole was heated at 70–75°C for an additional 3 h. It was poured into a two-layer mixture of ice-water (100 ml) and AcOEt (100 ml). The organic layer was separated, washed successively with water and saturated brine, and dried over $MgSO_4$. The organic layer was evaporated *in vacuo* to afford an oil, which was used in the next reaction without further purification. A mixture of an oil, sodium hydroxide (2.6 g, 60 mmol), water (100 ml), and MeOH (45 ml) was heated at 50–55°C for 3 h. The reaction mixture was then acidified to pH 5–6 with concentrated hydrochloric acid, and extracted with $CHCl_3$ (2×100 ml). The organic extracts were dried over $MgSO_4$ and evaporated under reduced pressure to afford a white solid. The resulting solid was recrystallized from EtOH–iPE to give **16a** (3.9 g, 50%) as colorless crystals, mp 117–119°C. 1H -NMR ($DMSO-d_6$) δ : 2.95 (3H, s), 3.40 (2H, t, $J=4$ Hz), 4.51 (2H, t, $J=4$ Hz), 6.85 (1H, d, $J=2$ Hz), 6.99 (1H, d, $J=6$ Hz), 7.46 (1H, dd, $J=2, 6$ Hz). *Anal.* Calcd for $C_{10}H_{11}NO_3$: C, 62.17; H, 5.74; N, 7.25. Found: C, 62.03; H, 5.72; N, 7.22. MS m/z : 193 (M^+).

Compounds **16b–d** were prepared by the same procedure as described for **16a**.

3,4-Dihydro-6-fluoro-4-methyl-2H-1,4-benzoxazine-8-carboxylic Acid (16b) Yield 53%, mp 164–166°C (EtOH–iPE). 1H -NMR ($DMSO-d_6$) δ : 2.87 (3H, s), 3.30 (2H, t, $J=4$ Hz), 4.22 (2H, t, $J=4$ Hz), 6.57 (1H, dd, $J=2, 9$ Hz), 6.64 (1H, dd, $J=2, 9$ Hz). *Anal.* Calcd for $C_{10}H_{10}FNO_3$: C, 56.87; H, 4.77; N, 6.63. Found: C, 56.55; H, 4.64; N, 6.59. MS m/z : 211 (M^+).

6-Bromo-3,4-dihydro-4-methyl-2H-1,4-benzoxazine-8-carboxylic Acid (16c) Yield 55%, mp 192–194°C (EtOH–iPE). 1H -NMR ($DMSO-d_6$) δ : 2.86 (3H, s), 3.29 (2H, t, $J=4$ Hz), 4.25 (2H, t, $J=4$ Hz), 6.87 (1H, d, $J=2$ Hz), 6.94 (1H, d, $J=2$ Hz). *Anal.* Calcd for $C_{10}H_{10}BrNO_3$: C, 44.18; H, 3.70; N, 5.15. Found: C, 43.99; H, 3.67; N, 5.12. MS m/z : 271 (M^+).

3,4-Dihydro-4,6-dimethyl-2H-1,4-benzoxazine-8-carboxylic Acid (16d) Yield 51%, mp 120–121°C (EtOH–iPE). 1H -NMR ($DMSO-d_6$) δ : 2.18 (3H, s), 2.83 (3H, s), 3.24 (2H, t, $J=4$ Hz), 4.22 (2H, t, $J=4$ Hz), 6.63 (1H, d, $J=2$ Hz), 6.70 (1H, d, $J=2$ Hz). *Anal.* Calcd for $C_{11}H_{12}NO_3$: C, 63.76; H, 6.32; N, 6.76. Found: C, 63.70; H, 6.19; N, 6.49. MS m/z : 207 (M^+).

3,4-Dihydro-4-methyl-6-nitro-2H-1,4-benzoxazine-8-carboxylic Acid (16e) A mixture of HNO_3 (d 1.5, 2.8 g, 44 mmol) and concentrated H_2SO_4 (1.9 ml) was added to a solution of **16a** (7.7 g, 40 mmol) in concentrated H_2SO_4 (20 ml) with keeping below 0°C, and stirred at 0–5°C for an additional 1 h. The reaction mixture was poured onto ice and the precipitates were collected, washed with cold water and recrystallized from EtOH to afford 4.4 g (46%) of **16e** as pale yellow leaflets, mp 235°C (dec.). 1H -NMR ($DMSO-d_6$) δ : 2.96 (3H, s), 3.36 (2H, t, $J=4$ Hz), 4.38 (2H, t, $J=4$ Hz), 7.42 (1H, d, $J=2$ Hz), 7.85 (1H,

d, $J=2$ Hz). *Anal.* Calcd for $C_{10}H_{10}N_2O_5$: C, 50.42; H, 4.23; N, 11.76. Found: C, 50.17; H, 4.11; N, 11.52. MS m/z : 238 (M^+).

N-(1-Azabicyclo[2.2.2]oct-3-yl)-6-chloro-3,4-dihydro-2H-1,4-benzoxazine-8-carboxamide (13a) Physical and spectral data for compound **13a** are listed in Tables 2 and 5. Isobutyl chloroformate (1.3 ml, 10 mmol) was added to a mixture of the carboxylic acid **9** (2.5 g, 10 mmol), NEt_3 (1.4 ml, 10 mmol), and AcOEt (30 ml) at –10°C. The mixture was stirred below –5°C for 30 min and a solution of 3-amino-1-azabicyclo[2.2.2]octane (1.3 g, 10 mmol) in AcOEt (5 ml) was added with stirring at –10°C. Stirring was continued at –10°C for 30 min, and then at room temperature for 1 h, water was added and the resulting mixture was extracted with AcOEt. The extract was washed with water, dried over $MgSO_4$ and evaporated to dryness. The residue was recrystallized and converted to the hydrochloride in the usual manner.

General Procedure for the Preparation of N-(1-Azabicyclo[2.2.2]oct-3-yl)-3,4-dihydro-2H-1,4-benzoxazine-8-carboxamides (13b–j) and 17a–e Physical and spectral data of compounds **13b–j** and **17a–e** are listed in Tables 2, 3 and 5. Pivaloyl chloride (1.2 ml, 10 mmol) was added to a mixture of a carboxylic acid **12b–j** or **16a–e** (10 mmol), NEt_3 (1.4 ml, 10 mmol) and AcOEt (30 ml) at 0°C. The mixture was stirred below 5°C for 30 min, and a solution of 3-amino-1-azabicyclo[2.2.2]octane (1.3 g, 10 mmol) in AcOEt (5 ml) was added with stirring at 5°C. Stirring was continued at 5°C for 30 min and then at room temperature for 1 h, water was added and the resulting mixture was extracted with AcOEt. The extract was washed with water, dried over $MgSO_4$ and evaporated to dryness. The residue was recrystallized and converted to the hydrochloride, fumarate, maleate, or tartrate in the usual manner.

6-Amino-N-(1-azabicyclo[2.2.2]oct-3-yl)-3,4-dihydro-4-methyl-2H-1,4-benzoxazine-8-carboxamide Malate (17f) A mixture of **17e** (14 g, 30 mmol) and EtOH (250 ml) was hydrogenated over Raney Ni (3.0 g) at atmospheric pressure and room temperature for 5 h. The reaction mixture was filtered with suction through Celite and washed with EtOH. The filtrate was evaporated to give 10 g (82%) of **17f**, and was converted to the malate in the usual manner. Physical and spectral data for compounds **17f** are listed in Tables 3 and 5.

Enantiomers of N-(1-Azabicyclo[2.2.2]oct-3-yl)-3,4-dihydro-4-methyl-2H-1,4-benzoxazine-8-carboxamide Malate (13b) Pivaloyl chloride (1.2 ml, 10 mmol) was added to a mixture of a carboxylic acid **12b** (2.3 g, 10 mmol), NEt_3 (1.4 ml, 10 mmol) and AcOEt (30 ml) at 0°C. The mixture was stirred at below 5°C for 30 min, and a solution of (S)- or (R)-3-amino-1-azabicyclo[2.2.2]octane (1.3 g, 10 mmol) in AcOEt (5 ml) was added with stirring at 5°C. Stirring was continued at 5°C for 30 min and then at room temperature for 1 h, water was added and the resulting mixture was extracted with AcOEt. The extract was washed with water, dried over $MgSO_4$ and evaporated to dryness. The resulting solid was recrystallized from AcOEt to give (S)- or (R)-**13b**. Physical data for compounds (S)- or (R)-**13b** are listed in Table 4.

[3H]Granisetron Binding [3H]Granisetron binding assay was performed according to the method of Nelson and Thomas.¹³ Rat cerebral cortex was homogenized in 20 volumes of 0.32 M sucrose and the homogenate was centrifuged at 1000 $\times g$ for 10 min. The supernatant was centrifuged at 40000 $\times g$ for 15 min. The pellet was resuspended in 20 volumes of HEPES buffer (50 mM, pH 7.4) and the suspension was incubated at 37°C for 10 min, and then centrifuged at 40000 $\times g$ for 15 min. The pellet was washed and centrifuged (40000 $\times g$ for 15 min). The final pellet was resuspended in 30 volumes of HEPES buffer and used as tissue homogenate. The binding assay consisted of 50 μ l of [3H]granisetron (New England Nuclear), 50 μ l of a displacing drug and 900 μ l of tissue homogenate. Following a 30 min incubation at 25°C, the assay mixture was rapidly filtered under reduced pressure through Whatman GF/B glass filters which had been presoaked in 0.1% polyethyleneimine. Filters were washed immediately with 3 $\times$ 3 ml of ice-cold Tris–HCl buffer (50 mM, pH 7.4). ICS 205,930 (100 μ M) was used for the determination of nonspecific binding. IC_{50} values were determined from concentration-inhibition curves. K_i values were determined from the relationship $K_i = IC_{50} / (1 + c/K_d)$, where c is the concentration of [3H]granisetron and K_d is the dissociation constant of [3H]granisetron.

von Bezold-Jarisch Reflex Test The antagonism of 5-HT-induced bradycardia was evaluated according to the method of Fozard.¹⁴ Male Wistar rats weighing 350–450 g were anesthetized with urethane (1.25 g/kg i.p.). Blood pressure was recorded from the left femoral artery by means of a pressure transducer. Heart rate was monitored with a tachometer (San-ei, model 1321). The jugular vein was cannulated for intravenous injections of the test drugs and 5-HT. After the completion

of operative procedures, 100 units of heparin (Heparin sodium injection-N, Shimadzu) was injected intravenously. The test drug was administered 5 min before the rapid bolus injection of 5-HT, (10 or 20 $\mu\text{g/kg}$). For inhibition of 5-HT-induced changes in heart rate, statistical significance between mean values was determined by using Student's *t* test for paired data. The criterion of statistical significance was $p < 0.05$. The ED_{50} value of the test drug was determined by a modification of the method of Waud¹⁵⁾ as the dose which suppressed the bradycardia by 50%.

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