Design and Synthesis of 6-Chloro-3,4-dihydro-4-methyl-2*H*-1,4-benzoxazine-8-carboxamide Derivatives as Potent Serotonin-3 (5-HT_3) Receptor Antagonists

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Several 3-substituted 5-chloro-2-methoxybenzamides were synthesized and evaluated for serotonin-3 (5-HT_3) receptor binding affinity. The 5-HT_3 receptor antagonistic activity of zacopride, a representative 5-HT_3 receptor antagonist, was unchanged by the replacement of the 4-amino substituent on the aromatic moiety by a 3-dimethylamino substituent. This finding prompted a structural modification of azasetron, another 5-HT_3 receptor antagonist. Consequently, a new series of 3,4-dihydro-2*H*-1,4-benzoxazine-8-carboxamides was obtained and these compounds were found to be more potent than 3,4-dihydro-3-oxo-2*H*-1,4-benzoxazine-8-carboxamides. In particular, (*S*)-*N*-(1-azabicyclo[2.2.2]oct-3-yl)-6-chloro-3,4-dihydro-4-methyl-2*H*-1,4-benzoxazine-8-carboxamide showed a high affinity for 5-HT_3 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.

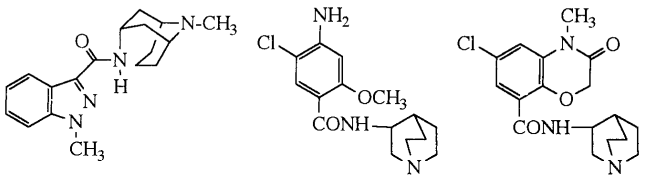
Key words 1,4-benzoxazine-8-carboxamide; 5-HT_3 receptor antagonist; structure–activity relationship; 5-HT_3 receptor binding; von Bezold-Jarisch reflex

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-HT_3 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-HT_3 receptor antagonists, because 5-HT_3 receptors have been identified in the peripheral and central nervous systems.[2a–d] Following the discovery of various 5-HT_3 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-HT_3 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.

Zacopride[5] is a typical member of the benzamide family. The 4-aminobenzamide structure has been reported to play an important role in the 5-HT_3 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-HT_3 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-2*H*-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-2*H*-1,4-benzoxazine-8-carboxamide derivatives as new 5-HT_3 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-



granisetron **zacopride** **azasetron**

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

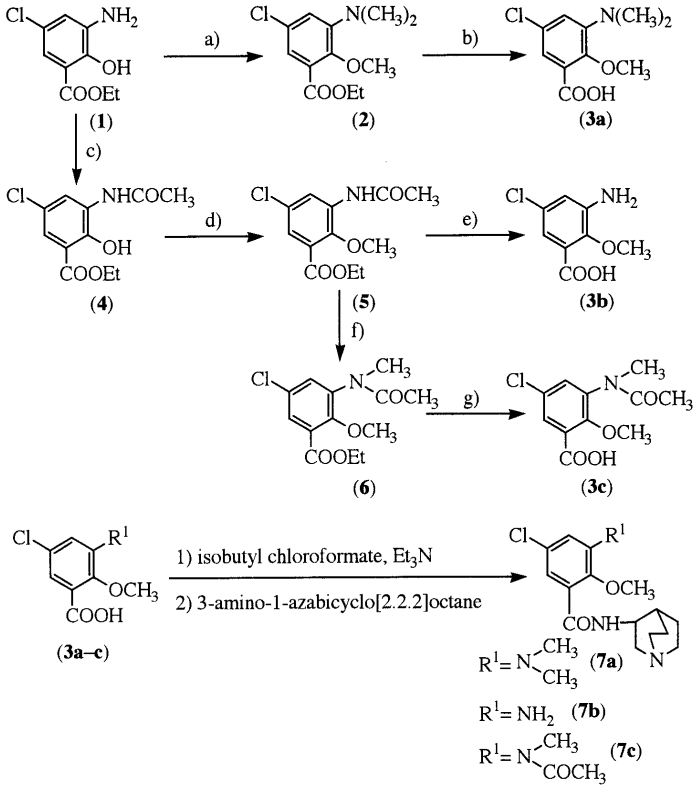
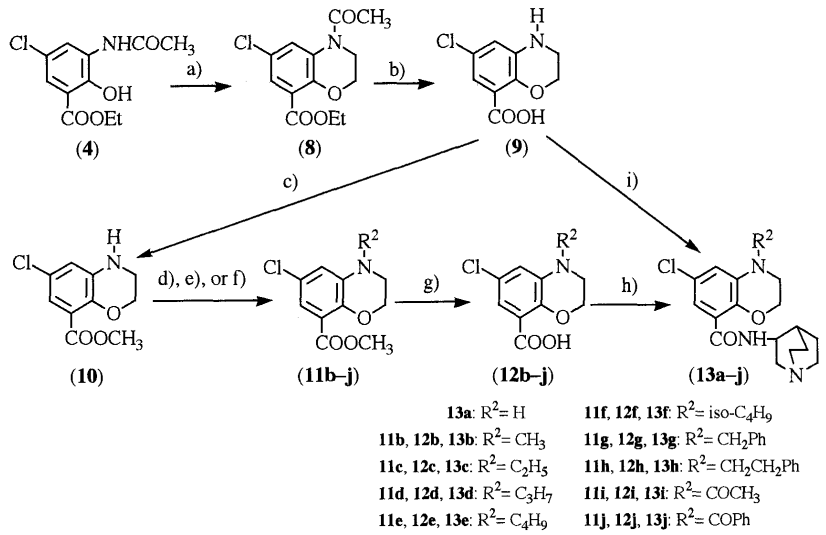


Chart 2

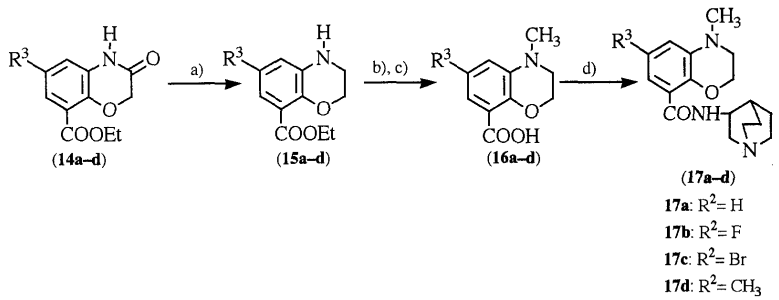
Table 1. 5-HT_3 Receptor Binding Affinity of Compounds **7a—c**

Compd. No.	mp (°C) (Recryst. solvent)	Yield (%)	Formula	Analysis (%) Calcd C	Calcd H	Calcd N	Found C	Found H	Found N	[^{3}H]Granisetron binding K_i (nM)
7a	159—160 (AcOEt)	61.2	$C_{17}H_{24}ClN_3O_2$	60.38	7.10	12.43	60.34	7.10	12.40	0.047
7b	192—194 (AcOEt)	25.8	$C_{15}H_{20}ClN_3O_2$	58.16	6.51	13.56	57.91	6.62	13.72	3.7
7c	186—188 (EtOH–acetone)	60.0	$C_{18}H_{24}ClN_3O_3 \cdot$ oxalate	52.64	5.70	9.21	52.32	5.65	9.13	22
Zacopride										0.18



13a: R^2= H
11b, 12b, 13b: R^2= CH_3
11c, 12c, 13c: R^2= C_2H_5
11d, 12d, 13d: R^2= C_3H_7
11e, 12e, 13e: R^2= C_4H_9
11f, 12f, 13f: R^2= iso-C_4H_9
11g, 12g, 13g: R^2= CH_2Ph
11h, 12h, 13h: R^2= CH_2CH_2Ph
11i, 12i, 13i: R^2= $COCH_3$
11j, 12j, 13j: R^2= COPh

Chart 3



17a: R^2= H
17b: R^2= F
17c: R^2= Br
17d: R^2= CH_3

Chart 4

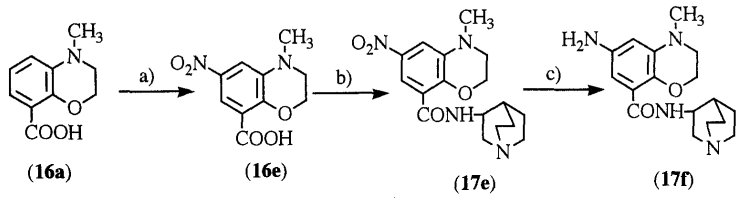


Chart 5

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.

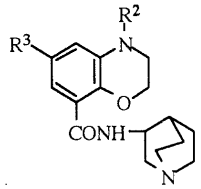
Pharmacological Results and Discussion

The 5-HT_3 receptor binding affinity of the synthesized compounds (**7a—c**, **13a—j** and **17a—f**) was determined by measurement of displacement of [^{3}H]granisetron binding in rat cerebrocortical membranes.[10] The 5-HT_3 receptor antagonistic activity was assessed in ferms 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-HT_3 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

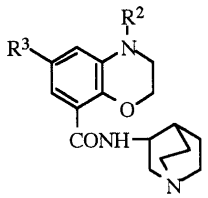
atom at position 4 of the 1,4-benzoxazine ring of azasetron is never basic, because of its amido structure. Therefore, we intended to change the amido moiety into an amino moiety. This idea led us to design **13a—j**, in which the aromatic moiety is a 6-chloro-3,4-dihydro-2*H*-1,4-benzoxazine ring. As we expected, compound **13b** was 7.5-fold more potent than azasetrone (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-HT_3 Receptor Binding Affinity of Compounds **13a—j**



Compd. No.	R^2	R^3	mp (°C) (Recryst. solvent)	Yield (%)	Formula	Analysis (%) Calcd (Found) C	H	N	[^{3}H]Granisetron binding K_i (nM)
13a	H	Cl	212—214 (EtOH)	19	$C_{16}H_{20}ClN_3O_2 \cdot 2HCl \cdot 1/2H_2O$	47.60 (47.73	5.74 5.47	10.41 10.10)	0.66
13b	CH_3	Cl	162 (dec.) (EtOH–AcOEt)	72	$C_{17}H_{22}ClN_3O_2 \cdot 2HCl \cdot 1/2H_2O$	48.88 (48.99	6.03 5.98	10.06 9.73)	0.072
13c	CH_2CH_3	Cl	209—211 (EtOH–acetone)	62	$C_{18}H_{24}ClN_3O_2 \cdot$ fumarate $\cdot 1/4H_2O$	56.17 (56.01	6.11 6.09	8.93 8.58)	0.3
13d	$CH_2CH_2CH_3$	Cl	212—214 (EtOH–acetone)	65	$C_{19}H_{26}ClN_3O_2 \cdot$ fumarate	57.25 (57.56	6.39 6.30	8.43 8.75)	1.8
13e	$CH_2CH_2CH_2CH_3$	Cl	193—194 (EtOH–acetone)	56	$C_{20}H_{28}ClN_3O_2 \cdot$ fumarate	57.98 (58.35	6.52 6.53	8.41 8.51)	1.8
13f	$CH_2CH(CH_3)_2$	Cl	204—206 (EtOH–acetone)	54	$C_{20}H_{28}ClN_3O_2 \cdot$ fumarate $\cdot 1/4H_2O$	57.83 (57.54	6.57 6.61	8.43 8.09)	6.8
13g	CH_2Ph	Cl	237—239 (EtOH)	38	$C_{23}H_{26}ClN_3O_2 \cdot$ fumarate $\cdot H_2O$	59.39 (60.37	5.91 5.77	7.70 7.59)	3.5
13h	CH_2CH_2Ph	Cl	185—186 (EtOH)	60	$C_{24}H_{28}ClN_3O_2 \cdot$ fumarate $\cdot H_2O$	60.05 (59.98	6.12 5.71	7.50) 6.95)	7.4
13i	$COCH_3$	Cl	155 (dec.) (EtOH)	34	$C_{18}H_{22}ClN_3O_3 \cdot HCl \cdot 2H_2O$	49.55 (49.30	6.24 5.73	9.63 9.21)	1.6
13j	COPh	Cl	207 (dec.) (IPA)	61	$C_{23}H_{24}ClN_3O_3 \cdot$ tartrate $\cdot H_2O$	54.78 (54.99	5.11 5.07	7.10 7.21)	12
Azasetron									0.54

Table 3. 5-HT_3 Receptor Binding Affinity of Compounds **17a—f**



Compd. No.	R^2	R^3	mp (°C) (Recryst. solvent)	Yield (%)	Formula	Analysis (%) Calcd (Found) C	H	N	[^{3}H]Granisetron binding K_i (nM)
17a	CH_3	H	178—180 (EtOH–acetone)	43	$C_{17}H_{23}N_3O_2 \cdot$ fumarate $\cdot 1/4H_2O$	59.78 (59.79	6.57 6.75	9.96 10.18)	0.56
17b	CH_3	F	201 (dec.) (EtOH–acetone)	75	$C_{17}H_{22}FN_3O_2 \cdot$ fumarate $\cdot 1/4H_2O$	57.33 (57.39	6.07 6.05	9.55 9.41)	0.14
17c	CH_3	Br	198 (dec.) (EtOH)	63	$C_{17}H_{22}BrN_3O_2 \cdot$ tartrate $\cdot 1/4H_2O$	47.34 (47.24	5.10 5.28	7.89 7.84)	0.15
17d	CH_3	CH_3	234 (dec.) (EtOH–acetone)	28	$C_{18}H_{25}N_3O_2 \cdot 1/2$fumarate $\cdot 1/4H_2O$	63.56 (63.40	7.33 7.40	11.12 11.11)	4.1
17e	CH_3	NO_2	224—226 (AcOEt)	63	$C_{17}H_{22}N_4O_4$	58.95 (58.67	6.40 6.34	16.17 16.02)	15
17f	CH_3	NH_2	148—149 (EtOH–acetone)	73	$C_{17}H_{24}N_4O_2 \cdot 3$maleate $\cdot 1/4H_2O$	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-HT_3 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-HT_3 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-HT_3 Receptor Antagonistic Activities of Compound **13b** and Its Enantiomers

Compd. No.	mp (°C) (Recryst. solvent)	Yield (%)	Formula	Analysis (%) Calcd (Found) C	H	N	[^{3}H]Granisetron binding K_i (nM)	BJ reflex[a] ED_{50} (μg/kg i.v.)
13b							0.072	0.28 (0.21—0.34)
(*S*)-**13b**	185—186[b] (AcOEt)	74	$C_{17}H_{22}ClN_3O_2$	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	$C_{17}H_{22}ClN_3O_2$	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. ^{1}H-NMR Spectral Data for **7a**—**c**, **13a**—**j**, and **17a**—**f** (100 MHz)

7a ($CDCl_3$)	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, br s)
7b ($CDCl_3$)	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, br s), 4.04—4.26 (1H, m), 6.81 (1H, d, $J=3$), 7.33 (1H, d, $J=3$), 7.70—8.00 (1H, br s)
7c (DMSO-d_6)	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, br s)
13a (DMSO-d_6)	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, br s), 6.69 (2H, s), 8.36—8.43 (1H, br s), 10.60—10.88 (1H, br s)
13b (DMSO-d_6)	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, br s), 10.53—10.64 (1H, br s)
13c (DMSO-d_6)	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, br s)
13d (DMSO-d_6)	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, br s)
13e (DMSO-d_6)	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, br s)
13f (DMSO-d_6)	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, br s)
13g (DMSO-d_6)	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, br s)
13h (DMSO-d_6)	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, br s)
13i (DMSO-d_6)	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, br s), 10.48—10.87 (1H, br s)
13j (DMSO-d_6)	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, br s)
17a (DMSO-d_6)	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, br s)
17b (DMSO-d_6)	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, br s)
17c (DMSO-d_6)	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, br s)
17d (DMSO-d_6)	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, br s)
17e (DMSO-d_6)	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, br s)
17f (DMSO-d_6)	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, br s), 6.12 (6H, s, maleate), 6.31 (1H, d, $J=2$), 6.43 (1H, d, $J=2$), 8.18—8.38 (1H, br s)

5-HT_3 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 ($ED_{50} = 0.089$ μg/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-HT_3 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-HT_3 receptors and potent antagonistic activity against the von Bezold-Jarisch reflex. The pharmacological superiority of **7a** and (*S*)-**13b** over their parent compounds suggestes that the basic nitrogen atom at position 3 of the benzamides contributes to the host-guest binding on the 5-HT_3 receptor.

Experimental

Melting points were determined in open capillaries and are uncorrected. Proton nuclear magnetic resonance (^{1}H-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-O1SG 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 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 (50 ml) and ethyl acetate (AcOEt, 50 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 chromatographed on silica gel to give **2** as an oil (4.2g, 71%). ^{1}H-NMR ($CDCl_3$) 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 ($CHCl_3$, 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 to give **3a** (2.0 g, 70%) as colorless crystals, mp 63—64 °C. ^{1}H-NMR ($CDCl_3$) 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 $C_{10}H_{12}ClNO_3$: 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), $CHCl_3$ (500 ml), and the mixture was aqueous saturated $NaHCO_3$ (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 $MgSO_4$. The organic layer was evaporated *in vacuo* to afford a solid, which was recrystallized from EtOH—diisopropyl ether (IPE) to give **4** (11g, 93%) as colorless crystals, mp 121—123 °C. ^{1}H-NMR ($CDCl_3$) 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 $C_{11}H_{12}ClNO_4$: 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 K_2CO_3 (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 $MgSO_4$. 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. ^{1}H-NMR ($CDCl_3$) 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 $C_{12}H_{14}ClNO_4$: 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 $CHCl_3$ (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 to give **3b** (2.4 g, 75%) as colorless crystals, mp 182—184 °C. ^{1}H-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 $C_8H_8ClNO_3 \cdot HCl \cdot 1/2H_2O$: 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 $MgSO_4$. 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%). ^{1}H-NMR ($CDCl_3$) 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*-methylacetylamino)benzoic 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. ^{1}H-NMR ($CDCl_3$) 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 $C_{11}H_{12}ClNO_4$: 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), 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-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 $MgSO_4$ 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. ^{1}H-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-2*H*-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 × 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. ^{1}H-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-2*H*-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. ^{1}H-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-2*H*-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 × 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. ^{1}H-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-2*H*-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 × 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. ^{1}H-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-2*H*-1,4-benzoxazine-8-carboxylic Acid (12d) Yield 45%, mp 136—137 °C (EtOH–IPE). ^{1}H-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-2*H*-1,4-benzoxazine-8-carboxylic Acid (12e) Yield 32%, mp 116—118 °C (EtOH–IPE). ^{1}H-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-2*H*-1,4-benzoxazine-8-carboxylic Acid (12f) Yield 49%, mp 124—125 °C (EtOH–IPE). ^{1}H-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-2*H*-1,4-benzoxazine-8-carboxylic Acid (12g) Yield 61%, mp 115—116 °C (EtOH–IPE). ^{1}H-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)-2*H*-1,4-benzoxazine-8-carboxylic Acid (12h) Yield 40%, mp 128—129 °C (EtOH–IPE). ^{1}H-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-2*H*-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 × 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. ^{1}H-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-2*H*-1,4-benzoxazine-8-carboxylic Acid (12j) Yield 63%, mp 175—176 °C (EtOH–IPE). ^{1}H-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-2*H*-1,4-benzoxazine-8-carboxylate (15a) Key intermediates **15a—d** were prepared from the amides **14a—d**[7] by Merkel's method[8] 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 borohydrate (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%). ^{1}H-NMR ($CDCl_3$) δ: 1.36 (3H, t, J=7 Hz), 3.44 (2H, t, J=4 Hz), 3.75—3.98 (1H, br s), 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-2*H*-1,4-benzoxazine-8-carboxylate (15b) Yield 83%, pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ: 1.36 (3H, t, J=7 Hz), 3.43 (2H, t, J=4 Hz), 3.95—4.18 (1H, br s), 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-2*H*-1,4-benzoxazine-8-carboxylate (15c) Yield 74%, pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ: 1.36 (3H, t, J=7 Hz), 3.42 (2H, t, J=4 Hz), 3.87—4.09 (1H, br s), 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-2*H*-1,4-benzoxazine-8-carboxylate (15d) Yield 81%, pale yellow oil. ^{1}H-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, br s), 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-2*H*-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. ^{1}H-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-2*H*-1,4-benzoxazine-8-carboxylic Acid (16b) Yield 53%, mp 164—166 °C (EtOH–IPE). ^{1}H-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-2*H*-1,4-benzoxazine-8-carboxylic Acid (16c) Yield 55%, mp 192—194 °C (EtOH–IPE). ^{1}H-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-2*H*-1,4-benzoxazine-8-carboxylic Acid (16d) Yield 51%, mp 120—121 °C (EtOH–IPE). ^{1}H-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-2*H*-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.). ^{1}H-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-2*H*-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-2*H*-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-2*H*-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-2*H*-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.

[^{3}H]Granisetron Binding [^{3}H]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×*g* for 10 min. The supernatant was centrifuged at 40000×*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×*g* for 15 min. The pellet was washed and centrifuged (40000×*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 μl of [^{3}H]granisetron (New England Nuclear), 50 μl of a displacing drug and 900 μl 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 3×3 ml of ice-cold Tris–HCl buffer (50 mM, pH 7.4). ICS 205,930 (100 μM) 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 [^{3}H]granisetron and K_d is the dissociation constant of [^{3}H]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 μg/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 Waud[15] as the dose which suppressed the bradycardia by 50%.

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