Benzoxazines. II.[1)]
Synthesis, Conformational Analysis, and Structure–Activity Relationships of 3,4-Dihydro-2*H*-1,4-benzoxazine-8-carboxamide Derivatives as Potent and Long-Acting Serotonin-3 (5-HT_3) Receptor Antagonists

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A series of 3,4-dihydro-2*H*-1,4-benzoxazine-8-carboxamide derivatives was synthesized and evaluated for serotonin-3 (5-HT_3) receptor antagonistic activities by means of assays of 5-HT_3 receptor binding and the ability to antagonize the von Bezold–Jarisch reflex in rats. Replacement of the 1,4-benzoxazine ring with a 1,4-benzthiazine ring or seven-membered ring (*i.e.*, 1,5-benzoxepine or 1,5-benzthiepine) resulted in decreased affinity for 5-HT_3 receptor. Introduction of substituents at the 2 position of the 1,4-benzoxazine ring increased the antagonistic activities (dimethyl > methyl > dihydro > phenyl). The compounds bearing a 9-methyl-9-azabicyclo[3.3.1]non-3-yl moiety as the basic part of 3,4-dihydro-2*H*-1,4-benzoxazine-8-carboxamide derivatives were equipotent to those bearing 1-azabicyclo[2.2.2]oct-3-yl moiety. The 9-methyl-9-azabicyclo[3.3.1]non-3-yl moiety was confirmed to adopt a boat–chair conformation on the basis of both NMR studies and X-ray analysis. In this series, *endo*-6-chloro-3,4-dihydro-*N*-(9-methyl-9-azabicyclo[3.3.1]non-3-yl)-2,2,4-trimethyl-2*H*-1,4-benzoxazine-8-carboxamide showed the highest affinity for 5-HT_3 receptors (K_i = 0.019 nM), and a long-lasting 5-HT_3 receptor antagonistic activity as evidenced by antagonism to the von Bezold–Jarisch reflex in rats. Such a long-lasting 5-HT_3 receptor antagonism would be attributed to the introduction of both two methyl groups at the 2 position of the benzoxazine ring and the 9-methyl-9-azabicyclo[3.3.1]non-3-yl moiety, which adopts the boat–chair conformation.

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

Nausea and vomiting are common side effects in the chemotherapeutic treatment of cancer, and much work has been done to develop effective and safe antiemetic agents to block these side effects. Selective 5-HT_3 receptor antagonists exhibit potent antagonism of chemotherapy- or radiation-induced emesis in human.[2)] Ondansetron,[3)] granisetron,[4)] and azasetron[5)] have already been marketed for this indication. A number of 5-HT_3 receptor antagonists have been reported from a decade. Further therapeutic indications such as the treatment of pain, migraine, dementia, anxiety, and drug abuse are being currently investigated.[6a–e)]

We previously reported that (*S*)-*N*-(1-azabicyclo[2.2.2]-oct-3-yl)-3,4-dihydro-2*H*-1,4-benzoxazine-8-carboxamide (**1**) is a potent 5-HT_3 receptor antagonist (Chart 1).[1)] In search of compounds with a superior pharmacological profile, we undertook structural modification of **1** by introducing of a 1,4-benzthiazine ring or a seven-membered ring (*i.e.*, 1,5-benzoxepine or 1,5-benzthiepine), in place of the 1,4-benzoxazine ring, introducing methyl or dimethyl or phenyl groups at the 2 position of the benzoxazine ring, and exchanging the 1-azabicyclo[2.2.2]-octane moiety for other basic components, (*i.e.*, 2-(*N*,*N*-diethylamino)ethyl, 1-benzyl-3-piperidinyl, 8-methyl-8-azabicyclo[3.2.1]oct-3-yl, or 9-methyl-9-azabicyclo-[3.3.1]non-3-yl moiety). In the present paper, we describe the synthesis and structure–activity relationships of the benzoxazine-8-carboxamide, benzthiazine-8-carboxamide, benzoxepine-9-carboxamide, and benzthiepine-9-carboxamide derivatives, and discuss the duration of 5-HT_3 receptor antagonistic activities of some of them. Based on the results of the conformational analyses, we will also discuss the three-dimensional structure–activity relationships of the 8-methyl-8-azabicyclo[3.2.1]oct-3-yl moiety of **27** and 9-methyl-9-azabicyclo[3.3.1]non-3-yl moiety of **28**.

Chemistry

7-Chloro-1,5-benzoxazine-9-carboxylic acid (**8**) was prepared in six steps from ethyl 3-acetamido-5-chlorosalicylate (**2**) as shown in Chart 2. Commercially available 5-chloro-2-hydroxybenzoic acid was converted into **2** in

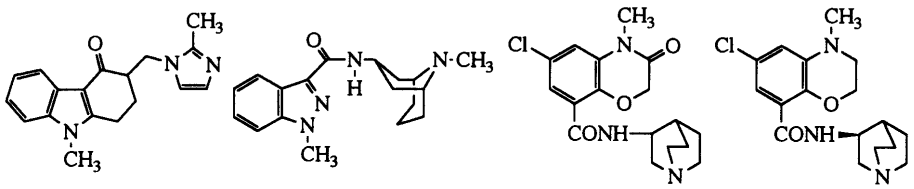

Chart 1

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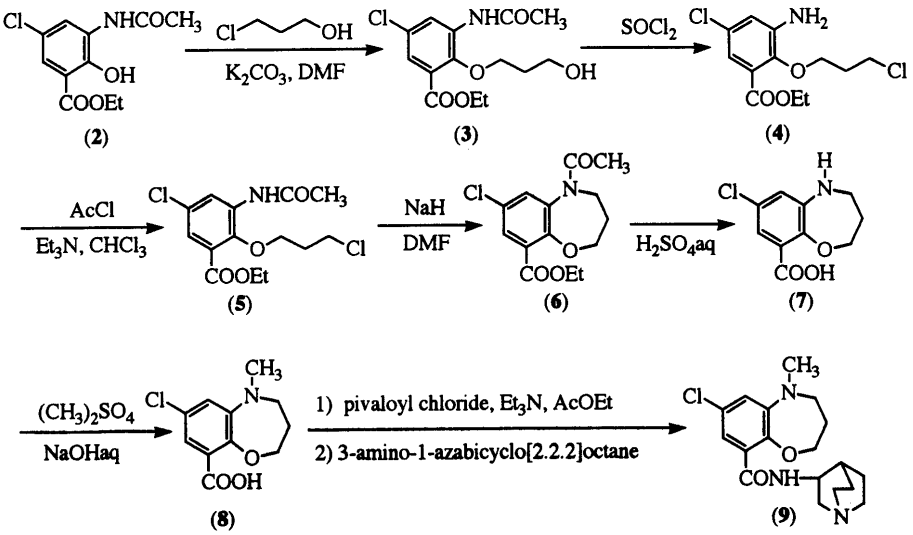


Chart 2

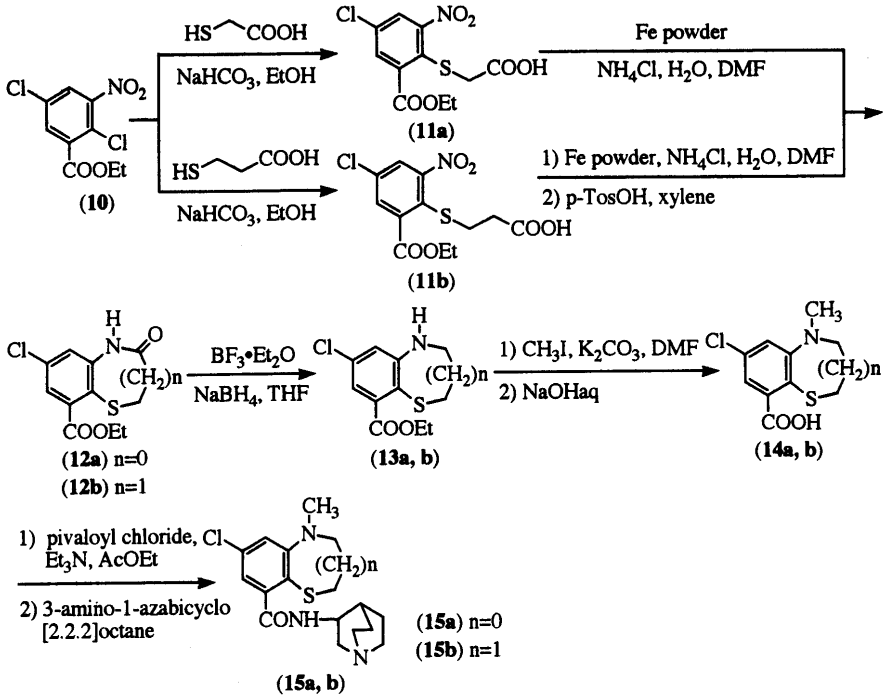


Chart 3

three steps as reported in the previous paper.[1] *O*-Alkylation of **2** with 3-chloropropanol to give **3**, followed by chlorination with thionyl chloride afforded **4** accompanied by *N*-deacetylation. An intermediate (**4**) was acetylated again with acetyl chloride. Cyclization of ethyl 3-acetamido-5-chloro-2-(3-chloropropyloxy)benzoate (**5**) by treatment with sodium hydride provided **6** having a desired ring system. Acid hydrolysis of **6** to give **7** followed by *N*-methylation with dimethyl sulfate under a basic condition provided **8**. Compound **9** was prepared from **8** by coupling of its mixed anhydride with 3-amino-1-azabicyclo[2.2.2]octane.

Benzthiazine and benzthiepine derivatives (**15a** and **15b**) were prepared as shown in Chart 3. Commercially available 2,5-dichlorobenzoic acid was converted into ethyl 2,5-dichloro-3-nitrobenzoate (**10**) by Spryskov's method.[7] From compound **10**, thioethers (**11a** and **11b**) were prepared by coupling with mercaptoacetic acid and 3-mercaptopropionic acid, respectively. The nitro group of **11a** was reduced with iron powder under a neutral condition, followed by spontaneous cyclization to afford **12a** with a desired ring system. Compound **11b** was reduced by the use of iron powder without spontaneous cyclization, and cyclization with an acid catalyst provided **12b**. The key intermediates (**13a** and **13b**) were prepared from amides (**12a** and **12b**, respectively) by Merkel's method,[8] which is convenient for the selective reduction of an amide moiety in presence of an ester moiety. Compounds **13a** and **13b** were methylated at the 5 position with iodomethane in the presence of K_2CO_3, followed by hydrolysis with base to afford carboxylic acids (**14a** and **14b**, respectively). Compounds **14a** and **14b** were coupled

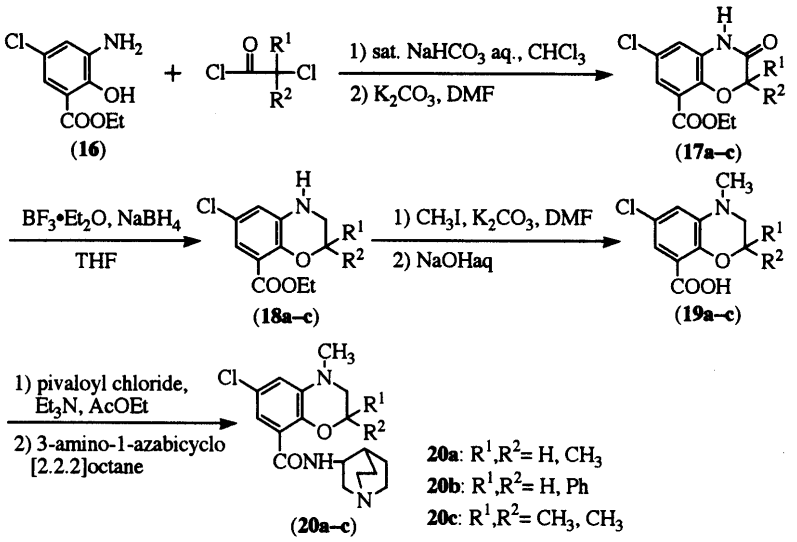


Chart 4

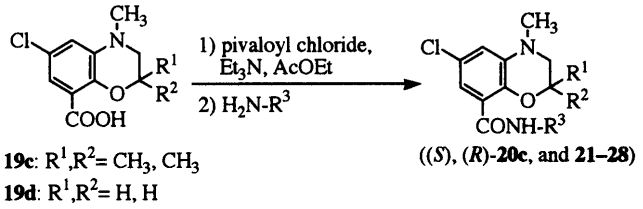


Chart 5

with 3-amino-1-azabicyclo[2.2.2]octane to give **15a** and **15b**, respectively.

The general route for the synthesis of the 2-substituted-1,4-benzoxazine-8-carboxamide derivatives (**20a—c**) is shown in Chart 4. Key intermediates **18a—c** were prepared from the amides **17a—c** by Merkel's method.[8] Compounds **18a—c** were methylated at the 4 position with iodomethane in the presence of K_2CO_3, followed by hydrolysis with base to afford the carboxylic acids **19a—c**. Compounds **19a—c** were coupled with 3-amino-1-azabicyclo[2.2.2]octane to give **20a—c**.

Compounds **21—28**, with altered basic side chains, were prepared as shown in Chart 5. The amides **21—28** were prepared from the carboxylic acid **19c** or **19d**[1] by coupling with appropriate amines *via* mixed anhydrides. Each enantiomer of **20c** was prepared by coupling the carboxylic acid **19c** with (*S*)- or (*R*)-3-amino-1-azabicyclo[2.2.2]-octane[9] in the same manner as shown in Chart 5.

Results and Discussion

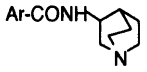
The 5-HT_3 receptor binding affinity of the synthesized compounds **9**, **15a**, **15b**, **20a—c**, and **21—28** was determined by measurement of displacement of [^{3}H]granisetron binding in rat cerebrocortical membranes.[10] The 5-HT_3 receptor antagonistic activity was assessed in terms of the ability to inhibit the 5-HT-induced bradycardia (von Bezold–Jarisch reflex)[11] in rats as shown in Tables 1 and 2.

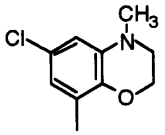
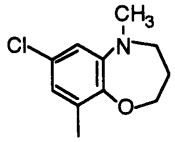
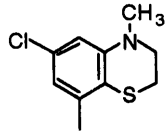
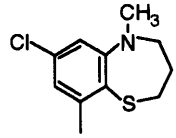
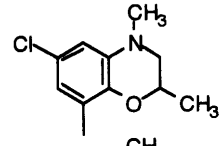
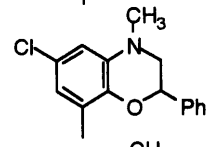
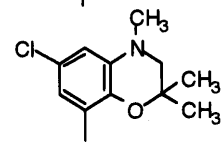
The replacement of the 1,4-benzoxazine ring of **1** by a 1,5-benzoxazepine ring resulted in a decreased affinity (**1** *vs.* **9**), and the compound **9** was also a short-acting antagonist (Table 3). The 1,4-benzthiazine derivative **15a** and 1,5-benzthiazepine derivative **15b** were 20 and 30 times less potent than the parent compound **1**. Replacement of the oxygen atom in both 1,4-benzoxazine ring and 1,5-benzoxazepine ring with a sulfur atom decreased the affinity, suggesting that a conformational restraint imposed by an intramolecular hydrogen bond plays an important role in increasing the affinity. In this series the 6-membered ring analogs (**1** and **15a**) were more potent than the 7-membered ring analogs (**9** and **15b**, respectively). The 1,4-benzoxazine ring of **1** can be superimposed on the 1,5-benzoxazepine ring of **9** (Fig. 1). The minimum-energy conformations of the 1,4-benzoxazine ring and 1,5-benzoxazepine ring were generated from a three-dimensional rigid conformational search using SYBYL6.2.[12] Figure 3 shows that the 1,5-benzoxazepine ring (7-membered ring) deviates from the aromatic plane. These results suggest that the 7-membered ring causes greater steric hindrance in binding to the 5-HT_3 receptor than does the 6-membered ring.

We further prepared 2-substituted analogs, bearing a methyl, dimethyl, or phenyl group at the 2 position of the benzoxazine ring. Among them, the 2-methyl compound **20a** and 2,2-dimethyl compound **20c** were slightly more potent than **1**, although the 2-phenyl compound **20b** was less potent than **1**. This result indicates that substitution at the **2** position of the 1,4-benzoxazine ring causes increased steric hindrance in binding to the 5-HT_3 receptor.

Because compound **20c** has a chiral center on its quinuclidine ring, this compound consists of two enantiomers. Thus, we synthesized the (*S*)- and (*R*)-enantiomer of **20c** to compare their pharmacological activities. The (*S*)-enantiomer of **20c** was equipotent to its counterpart, although the (*S*)-enantiomers of the *N*-(1-azabicyclo[2.2.2]oct-3-yl)benzamide derivatives, including zacopride[13] and compound **1**,[1] are one order of magnitude more potent than the (*R*)-enantiomers. The (*S*)-enantiomer of **20c** inhibited the 5-HT-induced bradycardia *in vivo* in anesthetized rats after intravenous administration with a moderate duration of action (Table 3).

Table 1. 5-HT_3 Receptor Binding Affinity of Compounds **1**, **9**, **16a**, **16b**, and **20a—b**



Compd. No.	Ar	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.)
1								0.051	0.089 (0.074—0.10)
9		237 (dec.) EtOH	40	$C_{18}H_{24}N_3O_2Cl \cdot 2HCl \cdot 1/4H_2O$	50.59 (50.56	6.25 6.31	9.83 9.45)	0.45	0.35 (0.31—0.57)
15a		195—196 AcOEt	50	$C_{17}H_{22}N_3OSCl \cdot 1/2H_2O$	56.57 (56.97	6.42 6.27	11.64 11.53)	15	NT[b]
15b		216 (dec.) EtOH	67	$C_{18}H_{24}N_3OSCl \cdot 2HCl \cdot H_2O$	47.32 (47.67	6.18 6.00	9.20 9.09)	21	NT[b]
20a		283 (dec.) EtOH	53	$C_{18}H_{24}N_3O_2Cl \cdot HCl \cdot 1/2H_2O$	54.69 (54.50	6.63 6.43	10.63 10.56)	0.041	0.23 (0.14—0.32)
20b		194—196 EtOH	61	$C_{23}H_{26}N_3O_2Cl \cdot HCl$	67.00 (66.84	6.55 6.43	10.19 10.12)	0.12	1.19 (1.12—1.28)
20c		216 (dec.) EtOH	60	$C_{19}H_{26}N_3O_2Cl \cdot HCl \cdot 1/4H_2O$	56.36 (56.26	6.85 6.68	10.38 10.42)	0.045	0.12 (0.11—0.18)

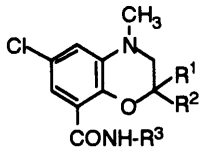
a) Serotonin was administered at a dose of 10 μg/kg i.v. 5 min posttreatment with a drug at the specified dose. Values in parentheses indicate the 95% confidence limits. *b*) Not tested.

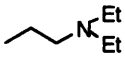
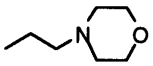
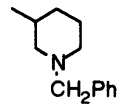
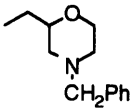
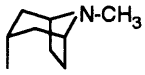
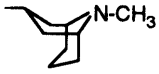
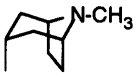
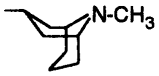
In order to investigate the effect of the basic side chain on duration of action, compounds bearing a simple or bicyclic amine as the basic side chain were prepared. None of compounds **21**—**24** bearing a simple amino moiety were potent 5-HT_3 receptor ligands. However, introduction of a bulkier amine (8-methyl-8-azabicyclo[3.2.1]oct-3-yl moiety or 9-methyl-9-azabicyclo[3.3.1]non-3-yl moiety) as the basic moiety resulted in a remarkable increase of the activity. Compounds **26** and **28** bearing a 9-methyl-9-azabicyclo[3.3.1]non-3-yl moiety exhibited an affinity comparable to that of **1**, and showed a long-lasting inhibition of 5-HT-induced bradycardia (Table 3).

To understand the pharmacological difference between **26** and **28**, and between **25** and **27**, it is important to know the three-dimensional structures of their amine moieties. It is well known that the six-membered ring of 3-substituted *endo*-8-methyl-8-azabicyclo[3.2.1]octane adopts a flattened chair conformation.[14a-f] On the other hand, there are four possible conformers (*i.e.*, chair–chair, chair–boat, boat–chair, and boat–boat types) for 3-substituted *endo*-9-methyl-9-azabicyclo[3.3.1]nonane. Recently, Fernandez *et al.* reported that *endo*-3-(*N*-substituted-amino)-9-phenethyl-9-azabicyclo[3.3.1]nonane adopts a boat–chair conformation on the basis of NMR studies.[15] Thus, the conformations of the azacycles of **25** and **27** would be quite different from those of **26** and **28**. To confirm this, we studied the solution structures of **27** and **28** by NMR spectroscopy.

Compounds **27**·HCl and **28**·HCl gave complicated NMR spectra, in which there are two sets of signals for each proton due to the inversion of protonated nitrogen in the azacycles, in deuteriodimethyl sulfoxide (DMSO-

Table 2. 5-HT_3 Receptor Binding Affinity of Compounds (*S*)-**20C**, (*R*)-**20c**, and **21a—h**



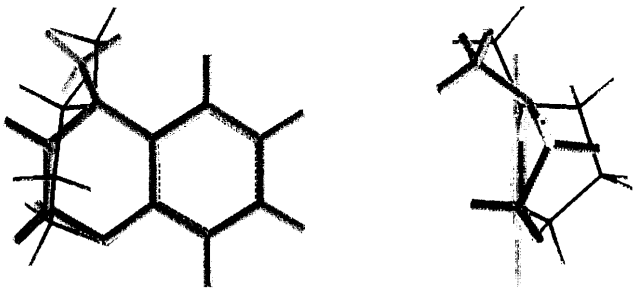
Compd. No.	R^1	R^2	R^3	mp (°C) (Recryst. solvent)	Yield (%)	Formula	Analysis (%) Calcd (Found) C	H	N	[^{3}H]Granisetron binding K_i (nM)	BJ reflex[a] ED_{50} (μg/kg i.v.)
(*S*)-**20c**[b]	CH_3	CH_3		273 (dec.) EtOH	62	$C_{19}H_{26}N_3O_2Cl \cdot HCl \cdot 1/4H_2O$	56.36 (56.25	6.85 6.61	10.38 10.45)	0.041	0.11 (0.097—0.12)
(*R*)-**20c**[c]	CH_3	CH_3		273 (dec.) EtOH	63	$C_{19}H_{26}N_3O_2Cl \cdot HCl \cdot 1/4H_2O$	56.36 (56.16	6.85 6.64	10.38 10.31)	0.045	0.2 (0.21—0.34)
21	H	H		195—197 IPA–IPE	46	$C_{16}H_{24}N_3O_2Cl \cdot HCl$	53.04 (53.08	6.67 6.81	11.60 11.52)	190	NT[d]
22	H	H		190—191 EtOH–acetone	49	$C_{16}H_{22}N_3O_3Cl \cdot 2HCl \cdot 1/2H_2O$	45.57 (45.87	5.97 5.99	9.96 9.85)	>1000	NT[d]
23	H	H		109—110 EtOH	61	$C_{22}H_{26}N_3O_2Cl$	66.07 (66.26	6.55 6.55	10.51 10.52)	99	NT[d]
24	H	H		154—156 EtOH–acetone	45	$C_{22}H_{26}N_3O_3Cl \cdot 2HCl \cdot 1/2H_2O$	53.03 (53.14	5.87 5.81	8.44 8.59)	>1000	NT[d]
25	H	H		183—185 IPA	71	$C_{18}H_{24}N_3O_2Cl$	61.80 (61.85	6.91 6.96	12.01 11.95)	0.27	0.47 (0.36—0.60)
26	H	H		159—161 IPA–acetone	63	$C_{19}H_{26}N_3O_3Cl \cdot HCl$	57.00 (56.98	6.80 6.52	10.50 10.47)	0.042	0.24 (0.21—0.27)
27	CH_3	CH_3		289 (dec.) EtOH	59	$C_{20}H_{28}N_3O_2Cl \cdot HCl \cdot 1/4H_2O$	57.35 (57.11	7.10 6.98	10.03 9.88)	0.20	0.43 (0.42—0.45)
28	CH_3	CH_3		262 (dec.) EtOH	81	$C_{21}H_{30}N_3O_2Cl \cdot HCl$	58.81 (58.73	7.23 7.09	9.80 9.84)	0.019	0.24 (0.21—0.27)

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*) $[\alpha] = +3.4$ ($c = 1.0$ H_2O). *c*) $[\alpha] = -3.4$ ($c = 1.0$ H_2O). *d*) Not tested.

d_6). Thus, we firstly studied the free bases of **27** and **28** in deuteriomethanol. The assignments of proton resonances were made by means of 2D correlation spectroscopy (COSY) experiments. The characteristic signals of H(3) of both compounds, in which the couplings with amide protons are decoupled for clarity, are shown in Fig. 2. The triplet pattern of the H(3) signal of **27** ($J = 7.1$ Hz) suggests that H(3) is an equatorial-oriented proton and the ring has a flattened chair conformation. On the other hand, the H(3) signal of **28** exhibits a triplet of triplet pattern ($J = 10.5$, 6.9 Hz). This indicates that the H(3) is an axial-oriented proton and the disubstituted ring has a boat conformation. The large coupling constant of 10.6 Hz for J H(1—21) also suggests the boat conformation. Further, the coupling constants for both H(1—81), and H(1—82) are less than 2 Hz, indicating that the monosubstituted ring has a chair conformation. Thus, the amine part of **28** adopts a boat–chair conformation. The boat–chair structure of **28** were also confirmed by 2D nuclear Overhauser effect (NOE) experiments (Fig. 2). We explored the conformation of the 9-methyl-9-azabicyclo-[3.3.1]non-3-yl moiety of granisetron, because no conformational studies of granisetron have been reported. By use of similar procedures, the 9-methyl-9-azabicyclo-[3.3.1]non-3-yl moiety of granisetron was also confirmed to adopt boat–chair conformation in its solution, although a number of 5-HT_3 receptor antagonists have been superimposed on granisetron with the an azacycle in

Table 3. Duration of Action of 5-HT_3 Receptor Antagonists after Intravenous Administration to Rats

Compound No.	BJ reflex ED_{50} (μg/kg i.v.) *vs.* time (min)						
	5	15	30	45	60	120	180
1	0.089 (0.074—0.10)	0.16 (0.13—0.21)	0.29 (0.23—0.35)	0.41 (0.33—0.53)	0.54 (0.44—0.66)	1.30 (1.04—1.67)	
9	0.35 (0.31—0.57)	0.47 (0.37—0.60)	1.04 (0.92—1.35)	1.46 (1.26—1.75)	2.25 (1.54—3.05)	6.88 (4.67—9.40)	
(*S*)-**20c**	0.11 (0.097—0.12)	0.09 (0.067—0.11)	0.08 (0.062—0.11)	0.11 (0.095—0.13)	0.11 (0.093—0.14)	0.32 (0.26—0.38)	
(*R*)-**20c**	0.17 (0.14—0.22)	0.16 (0.12—0.22)	0.23 (0.17—0.30)	0.41 (0.35—0.58)	0.48 (0.38—0.62)	1.08 (0.89—1.3)	
26	0.13 (0.11—0.17)	0.13 (0.11—0.16)	0.16 (0.11—0.23)	0.21 (0.13—0.29)	0.24 (0.21—0.29)		
28	0.24 (0.21—0.27)	0.15 (0.11—0.18)	0.11 (0.09—0.15)	0.10 (0.08—0.12)	0.11 (0.08—0.14)	0.12 (0.09—0.15)	0.21 (0.17—0.26)
Ondansetron	5.7 (4.4—7.2)	9.8 (6.4—13.6)	12.3 (10.2—20.5)	21.8 (16.0—25.5)	36.8 (31.5—81.1)		
Granisetron	0.74 (0.47—1.07)	2.67 (1.70—3.95)	4.09 (3.03—5.97)	7.23 (4.48—9.61)	8.44 (5.73—10.3)		
Azasetron	1.3 (0.9—2.0)	2.3 (1.7—3.2)	4.7 (3.0—7.2)	6.5 (4.1—10.0)	8.4 (5.7—11.0)		



Thick lines: 1,4-benzoxazine ring
Thin lines: 1,5-benzoxazepine ring

Fig. 1. Superimposition of the 1,4-Benzoxazine Ring and the 1,5-Benzoxazepine Ring

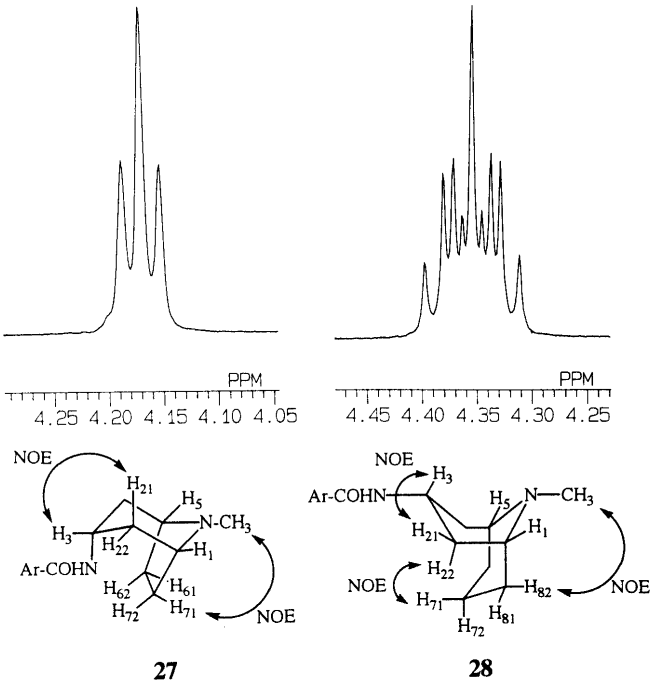


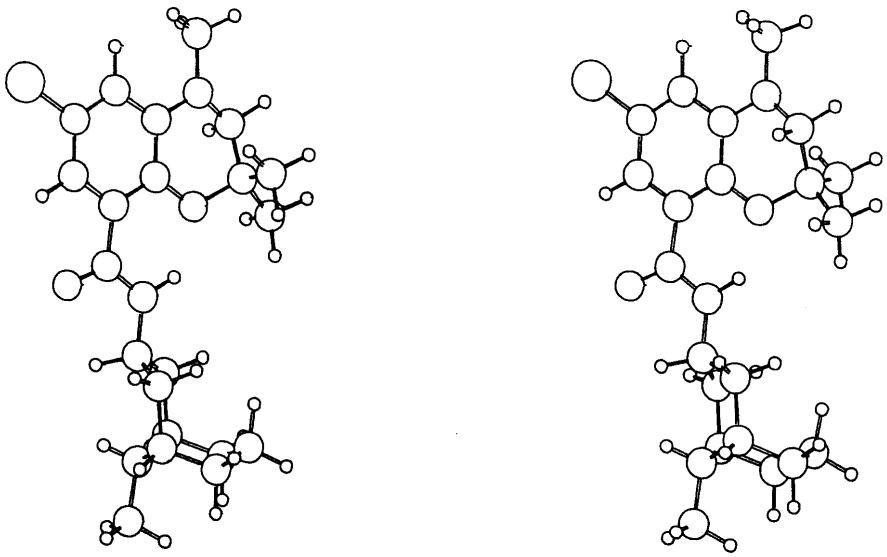
Fig. 2. ^{1}H-NMR Spectra of H(3) Regions of the Free Bases of **27** and **28**, and NOE Correlations of The Azacycles of the Free Bases of **27** and **28**

chair–chair conformation.

On the basis of the conformational analyses of free bases, those of compounds **27**·HCl and **28**·HCl were examined similarly. The coupling constants and NOE indicate that the conformations of both **27** and **28** are similar to those of their free bases. As regards the *N*-CH_3 groups of the azacycles of **27** and **28**, the preponderant *N*-CH_3 orientation is equatorial in DMSO-d_6. This result accords with the preponderance of equatorial *N*-CH_3 stereochemistry in the salts of tropane alkaloids.[16]

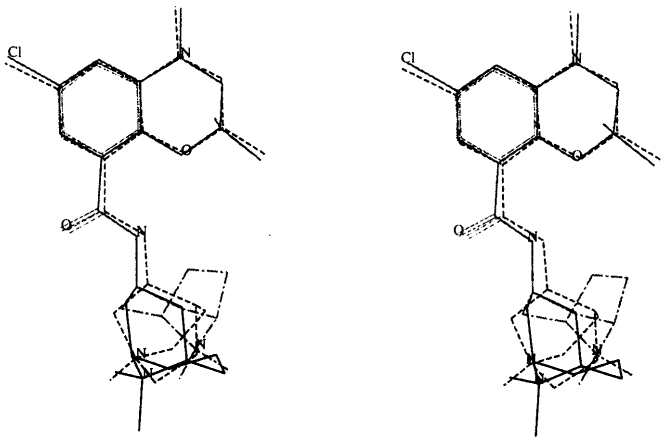
To establish the solid-state conformation, X-ray analysis of **28** has been carried out (Fig. 3).[17] There are two crystallographically independent molecules (A and B) in the crystal of **28**. As shown in Fig. 3, the 9-methyl-9-azabicyclo[3.3.1]non-3-yl moiety of both molecules adopts a boat–chair conformation, and the orientation of the 9-methyl group is equatorial.

Furthermore, to understand why **28** shows a potent and long-lasting 5-HT_3 receptor antagonism, we carried out superimposition of (*S*)-**20c** and **28**, together with **27** that showed a reduced affinity. The minimum-energy conformations of the three compounds were calculated with the "maximin 2" routine in the SYBYL6.2 software by use of the Tripos force field.[12] The three compounds can be superimposed, using the center of the aromatic ring, the heteroatoms of the amide moiety, and the basic nitrogen of the azacycle as the key points for the superimposition (Fig. 4). The front of the lone pair in 1-azabicyclo[2.2.2]oct-3-yl moiety of (*S*)-**20c** can be placed very close to that in the 9-methyl-9-azabicyclo[3.3.1]non-3-yl moiety of **28** without much strain energy. However, the front of the lone pair in the 8-methyl-8-azabicyclo[3.2.1]oct-3-yl moiety of **27** does not fit that in the 9-methyl-9-azabicyclo[3.3.1]non-3-yl moiety of **28**. The pharmacological superiority of **26** and **28** over **25** and **27** suggest that the three-dimensional structure of



A B

Fig. 3. Molecular Structures of **28**



Dashed lines: (*S*)-**20c**
Dot-dashed lines: **27**
Solid lines: **28**

Fig. 4. Stereoview of Superimposition of (*S*)-**20c**, **27**, and **28**

the 9-methyl-9-azabicyclo[3.3.1]non-3-yl moiety contributes to the host–guest-binding ability on 5-HT_3 receptor.

Although compound **28** showed the highest affinity for 5-HT_3 receptors (K_i=0.019 nM), it showed less potent activity than **1** on the Bezold–Jarisch reflex. This result might be related to the late maximum inhibition of the 5-HT induced bradycardia in the case of **28**. We are unable to realize the reason why maximum inhibition of the bradycardia is delayed by about 45 min. A pharmacokinetic study of **28** is in progress.

In conclusion, we have found that *endo-N*-(9-methyl-9-azabicyclo[3.3.1]non-3-yl)-6-chloro-2,2,4-trimethyl-3,4-dihydro-2*H*-1,4-benzoxazine-8-carboxamide (**28**) shows a potent and long-lasting 5-HT_3 receptor antagonism as evaluated using the von Bezold–Jarisch reflex. Such a long-lasting 5-HT_3 receptor antagonism would be attributed to the introduction of both the dimethyl groups at the 2 position of the benzoxazine ring and the 9-methyl-9-azabicyclo[3.3.1]non-3-yl moiety that adopts a boat–chair conformation. Long-acting 5-HT_3 receptor antagonists should help to lessen the burden of treatment for cancer patients.

Experimental

Melting points were determined in open capillaries and are uncorrected. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on JEOL FX-100 and JEOL GSX-400 spectrometers and the chemical shifts were expressed in ppm downfield from tetramethylsilane as an internal standard. Low-resolution mass spectra (MS) were obtained with a JEOL DX-300 spectrometer. Optical rotations were obtained on a JASCO DIP-181 digital polarimeter. Elemental analyses and measurement of spectral data were performed in our laboratory. Column chromatography was carried out on E. Merck Silica gel 60, 70—230 mesh. Yields were not optimized. Ondansetron and granisetron were prepared in our laboratory by the reported methods.[18a,b]

Ethyl 3-Acetylamino-5-chloro-2-(3-hydroxypropyl)oxybenzoate (3) 3-Chloropropanol (24 g, 0.18 mol) was added to a mixture of **2**[5] (42 g, 0.16 mol), dimethylformamide (DMF, 300 ml), and K_2CO_3 (34 g, 0.24 mol) with stirring at room temperature, and the mixture was heated at 70—75 °C for an additional 4 h. The solvent was evaporated and then the residue was poured into a two-layer mixture of ice-water (500 ml) and ethyl acetate (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 an oil, which was chromatographed on silica gel to give **3** as an oil (45 g, 86%), ^{1}H-NMR ($CDCl_3$) δ: 1.40 (3H, t, J=6 Hz), 1.98—2.33 (2H, m), 2.41 (3H, s), 2.86—3.11 (1H, br s), 3.58—4.35 (4H, m), 4.38 (2H, q, J=6 Hz), 7.53 (1H, d, J=2 Hz), 8.67 (1H, d, J=2 Hz), 9.02—9.25 (1H, br s). MS m/z: 315 (M^+).

Ethyl 3-Amino-5-chloro-2-(3-chloropropyl)oxybenzoate (4) A mixture of **2** (8.7 g, 27 mmol), thionyl chloride (50 ml), and DMF (1 drop) was heated to reflux for 10 h and then cooled to room temperature. The mixture was poured into ice-water (100 ml) and then alkalized with K_2CO_3, and extracted with chloroform ($CHCl_3$, 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 **4** as a yellow oil (4.8 g, 60%). ^{1}H-NMR ($CDCl_3$) δ: 1.39 (3H, t, J=6 Hz), 2.02—2.40 (2H, m), 3.82 (2H, t, J=5 Hz), 3.80—3.98 (2H, br s), 4.08 (2H, t, J=5 Hz), 4.35 (2H, q, J=6 Hz), 6.84 (1H, d, J=2 Hz), 7.12 (1H, d, J=2 Hz). MS m/z: 291 (M^+).

Ethyl 3-Acetylamino-5-chloro-2-(3-chloropropyl)oxybenzoate (5) Acetyl chloride (1.5 g, 18 mmol) was added dropwise to a solution of **4** (4.8 g, 16 mmol), $CHCl_3$ (100 ml), and triethylamine (2.5 g, 24 mmol) with stirring at 0—10 °C, and the mixture was stirred for an additional 2 h, then poured into water (100 ml). The separated organic layer was washed with water, and dried over $MgSO_4$. The organic layer was evaporated *in vacuo* to afford an oil, which was chromatographed on silica gel to give **5** as a yellow oil (5.2 g, 95%). ^{1}H-NMR ($CDCl_3$) δ: 1.39 (3H, t,

$J=6$ Hz), 2.03—2.44 (2H, m), 2.30 (3H, s), 3.85 (2H, t, $J=5$ Hz), 4.13 (2H, t, $J=5$ Hz), 4.36 (2H, q, $J=6$ Hz), 7.58 (1H, d, $J=2$ Hz), 7.95—8.13 (1H, br s), 8.68 (1H, d, $J=2$ Hz). MS *m/z*: 333 (M^+).

Ethyl 5-Acetyl-7-chloro-2,3,4,5-tetrahydro-1,5-benzoxazepine-9-carboxylate (6) A solution of **4** (5.2 ml, 16 mmol) in anhydrous DMF (20 ml) was added to a suspension of sodium hydride (60% dispersion in mineral oil, 0.7 g, 17 mmol) in anhydrous DMF (30 ml) with stirring at 0—10 °C. The mixture was heated at 45 °C for 1 h and then cooled to room temperature. The solvent was evaporated, then the residue was taken up in water (100 ml) and the solution was washed with $CHCl_3$ (100 ml). The separated organic layer was washed with water, 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 (3.4 g, 73%). ^{1}H-NMR ($CDCl_3$) δ: 1.38 (3H, t, $J=6$ Hz), 1.98 (3H, s), 2.00—2.44 (2H, m), 2.28—2.45 (2H, m), 4.02—4.43 (2H, m), 4.40 (2H, q, $J=6$ Hz), 7.43 (1H, d, $J=2$ Hz), 7.72 (1H, d, $J=2$ Hz). MS *m/z*: 297 (M^+).

7-Chloro-2,3,4,5-tetrahydro-1,5-benzoxazepine-9-carboxylic Acid (7) A mixture of **6** (2.3 g, 9 mmol), concentrated sulfuric acid (10 ml) and water (50 ml) was refluxed for 5 h. Further water (100 ml) was added, and the reaction mixture was adjusted to pH 3.5 with 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 a solid. The resulting solid was recrystallized from EtOH to give **7** (1.6 g, 75%) as pale yellow crystals, mp 188—189 °C. ^{1}H-NMR (DMSO-d_6) δ: 2.00—2.28 (2H, m), 3.39 (2H, t, $J=5$ Hz), 4.41 (2H, t, $J=5$ Hz), 6.90 (1H, d, $J=2$ Hz), 7.59 (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, 52.52; H, 4.41; N, 6.14. MS *m/z*: 227 (M^+).

7-Chloro-5-methyl-2,3,4,5-tetrahydro-1,5-benzoxazepine-9-carboxylic Acid (8) Dimethyl sulfate (0.53 g, 4 mmol) was added to a mixture of **7** (1.6 g, 7 mmol), water (30 ml), and sodium hydroxide (0.7 g, 18 mmol) under ice-cooling. Further dimethyl sulfate (0.53 g, 4 mmol) was added, and the mixture was poured into a two-layer mixture of ice-water (50 ml) and AcOEt (50 ml). The organic extracts were dried over $MgSO_4$ and evaporated under reduced pressure to afford a solid. The resulting solid was recrystallized from EtOH to give **8** (1.6 g, 75%) as pale yellow crystals, mp 112—113 °C. ^{1}H-NMR (DMSO-d_6) δ: 2.00—2.28 (2H, m), 3.39 (2H, t, $J=5$ Hz), 4.41 (2H, t, $J=5$ Hz), 6.90 (1H, d, $J=2$ Hz), 7.59 (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, 54.61; H, 5.01; N, 5.72. MS *m/z*: 241 (M^+).

***N*-(1-Azabicyclo[2.2.2]oct-3-yl)-7-chloro-5-methyl-2,3,4,5-tetrahydro-1,5-benzoxazepine-9-carboxamide (9)** Pivaloyl chloride (0.54 ml, 4.0 mmol) was added to a mixture of the carboxylic acid **8** (0.9 g, 3.7 mmol), NEt_3 (1.0 ml, 7.4 mmol), and AcOEt (20 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 (0.6 g, 4.0 mmol) in AcOEt (5 ml) was added with stirring at −10 °C. Stirring was continued at −10 °C for 30 min and at room temperature for 1 h, then 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 converted to the hydrochloride in the usual manner, and recrystallized from EtOH to give **9** (0.63 g, 40%) as colorless crystals. ^{1}H-NMR (D_2O) δ: 1.82—2.22 (4H, m), 2.23—2.48 (3H, m), 3.24 (3H, s), 3.08—3.50 (5H, m), 3.60 (2H, t, $J=5$ Hz), 3.69—4.00 (1H, m), 4.22 (2H, t, $J=5$ Hz), 4.38—4.59 (1H, m), 7.58 (1H, d, $J=2$ Hz), 7.64 (1H, d, $J=2$ Hz). Physical data **9** are listed in Table 1.

(4-Chloro-2-ethoxycarbonyl-6-nitrophenyl)thioacetic Acid (11a) Mercaptoacetic acid (35 g, 0.38 mol) was added to a mixture of **10**[7] (143 g, 0.54 mol), EtOH (1000 ml), and $NaHCO_3$ (64 g, 0.76 mol) with stirring at room temperature, and the mixture was heated to reflux for an additional 2 h. The mixture was poured into a two-layer mixture of 2 N HCl (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 an oil, which was chromatographed on silica gel to give **11a** as a yellow oil (66 g, 64%). ^{1}H-NMR ($CDCl_3$) δ: 1.39 (3H, t, $J=7$ Hz), 3.78 (2H, s), 4.48 (2H, q, $J=7$ Hz), 7.86 (1H, d, $J=2$ Hz), 7.92 (1H, d, $J=2$ Hz), 10.12—10.40 (1H, br s). MS *m/z*: 319 (M^+).

3-(4-Chloro-2-ethoxycarbonyl-6-nitrophenyl)thiopropionic Acid (11b) 3-Mercaptopropionic acid (20 g, 0.19 mol) was added to a mixture of **10**[7] (70 g, 0.27 mol), EtOH (350 ml), and $NaHCO_3$ (34 g, 0.41 mol) with stirring at room temperature, and the mixture was heated to reflux for an additional 6 h. The mixture was poured into a two-layer mixture of 2 N HCl (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 an oil, which was chromatographed on silica gel to give **11b** as a yellow oil (30 g, 57%). ^{1}H-NMR ($CDCl_3$) 1.38 (3H, t, $J=7$ Hz), 2.67 (2H, t, $J=6$ Hz), 3.21 (2H, t, $J=6$ Hz), 4.48 (2H, q, $J=7$ Hz), 7.82 (2H, s), 10.84—11.18 (1H, br s). MS *m/z*: 333 (M^+).

Ethyl 6-Chloro-3,4-dihydro-3-oxo-2H-1,4-benzthiazine-8-carboxylate (12a) Fe powder (41.3 ml, 0.74 mol) was added to an aqueous 0.78 N NH_4Cl (115 ml) solution with stirring at 85 °C, followed by addition of a solution of **11a** (66 g, 0.2 mol) in DMF (200 ml) over 30 min. The mixture was heated at 85—90 °C for an additional 1 h, then filtered with suction through Celite. The organic layer was poured into ice-water to afford a solid, which was recrystallized from EtOH–isopropyl ether (IPE) to give **12a** (41 g, 73%) as pale yellow crystals, mp 223—225 °C. ^{1}H-NMR ($CDCl_3$) 1.39 (3H, t, $J=7$ Hz), 3.75 (2H, s), 4.48 (2H, q, $J=7$ Hz), 7.25 (1H, d, $J=2$ Hz), 7.40 (1H, d, $J=2$ Hz), 8.80—9.02 (1H, br s). *Anal.* Calcd for $C_{11}H_{10}ClNO_3S$: C, 48.62; H, 3.71; N, 5.15. Found: C, 48.51; H, 3.93; N, 4.87. MS *m/z*: 271 (M^+).

Ethyl 7-Chloro-4-oxo-2,3,4,5-tetrahydro-1,5-benzthiazepine-9-carboxylate (12b) Fe powder (18 g, 0.32 mol) was added to an aqueous 0.78 N NH_4Cl (50 ml) solution with stirring at 85 °C, followed by addition of a solution of **11b** (30 g, 0.09 mol) in DMF (100 ml) over 30 min. The mixture was heated at 85—90 °C for an additional 1h, then filtered with suction through Celite. The organic layer was poured into an ice-water to afford a solid. The solid and a catalytic amount of *p*-toluenesulfonic acid were dissolved in xylene (200 ml). Using a Dean–Stark trap, the solution was refluxed for 18 h. After cooling, the mixture was washed with saturated aqueous K_2CO_3 solution and concentrated. The residue was recrystallized from AcOEt to give **12b** (14 g, 56%) as pale yellow crystals, mp 179—180 °C. ^{1}H-NMR ($CDCl_3$) 1.41 (3H, t, $J=7$ Hz), 2.65 (2H, t, $J=6$ Hz), 3.44 (2H, t, $J=6$ Hz), 4.41 (2H, q, $J=7$ Hz), 7.25 (1H, d, $J=2$ Hz), 7.40 (1H, d, $J=2$ Hz), 8.80—9.02 (1H, br s). *Anal.* Calcd for $C_{12}H_{12}ClNO_3S$: C, 50.44; H, 4.23; N, 4.90. Found: C, 50.16; H, 4.16; N, 4.88. MS *m/z*: 285 (M^+).

Ethyl 6-Chloro-3,4-dihydro-2*H*-1,4-benzthiazine-8-carboxylate (13a) The key intermediate **13a** was prepared from the amide **12a** by Merkel's method.[8] Boron trifluoride diethyl etherate (10.3 ml, 84 mmol) was added dropwise to a mixture of **12a** (11 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 at 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 alkalized 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 a yellow solid. It was recrystallized from EtOH–IPE to give **13a** as pale yellow crystals (6.5 g, 79%), mp 105—106 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.36 (3H, t, $J=7$ Hz), 2.92 (2H, t, $J=5$ Hz), 3.63 (2H, t, $J=5$ Hz), 4.00—4.38 (1H, br s), 4.33 (2H, q, $J=7$ Hz), 6.56 (1H, d, $J=2$ Hz), 7.26 (1H, d, $J=8$ Hz). *Anal.* Calcd for $C_{11}H_{12}ClNO_2S$: C, 51.26; H, 4.69; N, 5.43. Found: C, 51.17; H, 4.63; N, 5.46. MS *m/z*: 257 (M^+).

Ethyl 7-Chloro-2,3,4,5-tetrahydro-1,5-benzthiazepine-9-carboxylate (13b) Compound **13b** was prepared by the same procedure as described for **13a**. Yield 92%, pale yellow crystals, mp 105—106. ^{1}H-NMR ($CDCl_3$) δ: 1.40 (3H, t, $J=7$ Hz), 2.00 (2H, tt, $J=5$, 5 Hz), 3.10 (2H, t, $J=5$ Hz), 3.64 (2H, t, $J=5$ Hz), 3.71—3.98 (1H, br s), 4.36 (2H, q, $J=7$ Hz), 6.63 (1H, d, $J=2$ Hz), 7.03 (1H, d, $J=8$ Hz). *Anal.* Calcd for $C_{12}H_{14}ClNO_2S$: C, 53.04; H, 5.19; N, 5.15. Found: C, 53.14; H, 5.24; N, 5.12. MS *m/z*: 271 (M^+).

6-Chloro-3,4-dihydro-4-methyl-2*H*-1,4-benzthiazine-8-carboxylic Acid (14a) Methyl iodide (5 ml, 80 mmol) was added to a mixture of **13a** (10 g, 40 mmol), dimethylformamide (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 an oil, which was used in the next reaction without further purification. A mixture of this 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 at reduced pressure to afford a white solid. The resulting solid was recrystallized from EtOH–IPE to give **14a** (5.1 g, 52%) as colorless crystals, mp 243—245 °C (dec.). ^{1}H-NMR ($CDCl_3$) δ: 3.00 (2H, t, J=4 Hz), 3.02 (3H, s), 3.65 (2H, t, J=4 Hz), 6.81 (1H, d, J=2 Hz), 7.43 (1H, d, J=2 Hz), 10.00—10.93 (1H, br s). *Anal.* Calcd for $C_{10}H_{10}ClNO_2S$: C, 49.28; H, 4.14; N, 5.75. Found: C, 49.34; H, 4.10; N, 5.77. MS *m/z*: 243 (M^+).

7-Chloro-5-methyl-2,3,4,5-tetrahydro-1,5-benzthiazepine-9-carboxylic Acid (14b) Compound **14b** was prepared by the same procedure as described for **14a**. Yield 53%, mp 171—172 °C (EtOH–IPE). ^{1}H-NMR ($CDCl_3$) δ: 2.00 (2H, tt, J=4, 4 Hz), 2.92 (3H, s), 3.05 (2H, t, J=4 Hz), 3.56 (2H, t, J=4 Hz), 6.84 (1H, d, J=2 Hz), 7.37 (1H, d, J=2 Hz), 10.02—10.88 (1H, br s). *Anal.* Calcd for $C_{12}H_{12}ClNO_2S$: C, 51.26; H, 4.69; N, 5.43. Found: C, 51.18; H, 4.63; N, 5.43. MS *m/z*: 257 (M^+).

Ethyl 6-Chloro-3,4-dihydro-2-methyl-3-oxo-2*H*-1,4-benzoxazine-8-carboxylate (17a) 2-Chloropropionyl chloride (8.4 g, 66 mmol) was added dropwise to a mixture of **16** (8.9 g, 44 mmol) in $CHCl_3$ (200 ml) and aqueous saturated $NaHCO_3$ (200 ml) with vigorous stirring at 0—10 °C and stirring was continued for an additional 1 h. The separated organic layer was washed with water, dried over $MgSO_4$ and evaporated at reduced pressure to afford a solid. The residue (methyl 5-chloro-3-(2-chloropropionyl)amino-2-hydroxybenzoate) was taken up in DMF, and K_2CO_3 was added. The whole was stirred at 70 °C for 2 h. After cooling, the mixture was poured into water, then extracted with $CHCl_3$. The 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 **17a** (6.7 g, 57%) as colorless crystals, mp 190—191 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.39 (3H, t, J=7 Hz), 1.62 (3H, d, J=7 Hz), 4.37 (2H, q, J=7 Hz), 4.76 (2H, q, J=7 Hz), 7.01 (1H, d, J=2 Hz), 7.48 (1H, d, J=2 Hz), 9.45—9.62 (1H, br s). *Anal.* Calcd for $C_{12}H_{12}ClNO_4$: C, 53.44; H, 4.48; N, 5.19. Found: C, 53.23; H, 4.46; N, 5.24. MS *m/z*: 269 (M^+).

Ethyl 6-Chloro-3,4-dihydro-3-oxo-2-phenyl-2*H*-1,4-benzoxazine-8-carboxylate (17b) Compounds **17b,c** were prepared by the same procedure as described for **17a**. Yield 38%, colorless crystals, mp 164—165 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.37 (3H, t, J=7 Hz), 4.39 (2H, q, J=7 Hz), 5.88 (1H, s), 6.96 (1H, d, J=2 Hz), 7.22—7.59 (5H, m), 7.51 (1H, d, J=2 Hz). MS *m/z*: 331 (M^+).

Ethyl 6-Chloro-3,4-dihydro-2,2-dimethyl-3-oxo-2*H*-1,4-benzoxazine-8-carboxylate (17c) Yield 51%, colorless crystals, mp 164—165 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.38 (3H, t, J=7 Hz), 1.58 (6H, s), 4.41 (2H, q, J=7 Hz), 7.05 (1H, d, J=2 Hz), 7.57 (1H, d, J=2 Hz), 9.39—9.61 (1H, br s). *Anal.* Calcd for $C_{13}H_{14}ClNO_4$: C, 55.04; H, 4.97; N, 4.94. Found: C, 54.99; H, 4.96; N, 4.91. MS *m/z*: 283 (M^+).

Ethyl 6-Chloro-3,4-dihydro-2-methyl-2*H*-1,4-benzoxazine-8-carboxylate (18a) Compounds **18a—c** were prepared from the amides **17a—c**, respectively, by the same procedure as described for **13a**. Yield 77%, pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ: 1.37 (3H, t, J=7 Hz), 1.41 (3H, d, J=6 Hz), 2.98—3.48 (2H, m), 3.81—4.04 (1H, br s), 4.10—4.42 (1H, m), 4.34 (2H, q, J=7 Hz), 6.65 (1H, d, J=Hz), 7.07 (1H, d, J=2 Hz). MS *m/z*: 255 (M^+).

Ethyl 6-Chloro-3,4-dihydro-2-phenyl-2*H*-1,4-benzoxazine-8-carboxylate (18b) Yield 68%, pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ: 1.36 (3H, t, J=7 Hz), 3.19—3.72 (2H, m), 3.92—4.30 (1H, br s), 4.32 (2H, q, J=7 Hz), 5.05—5.24 (1H, m), 6.76 (1H, d, J=2 Hz), 7.18 (1H, d, J=2 Hz), 7.27—7.58 (5H, m). MS *m/z*: 317 (M^+).

Ethyl 6-Chloro-3,4-dihydro-2,2-dimethyl-2*H*-1,4-benzoxazine-8-carboxylate (18c) Yield 91%, pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ: 1.36 (3H, t, J=7 Hz), 1.36 (6H, s), 3.11 (2H, s), 3.42—3.94 (1H, br s), 4.31 (2H, q, J=7 Hz), 6.65 (1H, d, J=2 Hz), 7.09 (1H, d, J=2 Hz). MS *m/z*: 269 (M^+).

6-Chloro-3,4-dihydro-2,4-dimethyl-2*H*-1,4-benzoxazine-8-carboxylic Acid (19a) Compounds **19a—c** were prepared from **18a—c**, respectively, by the same procedure as described for **14a**. Yield 72%, mp 158—159 °C (EtOH). ^{1}H-NMR (DMSO-d_6) δ: 1.28 (3H, d, J=4 Hz), 2.85 (3H, s), 3.10—3.42 (2H, m), 4.08—4.43 (1H, m), 6.72 (1H, d, J=2 Hz), 6.78 (1H, d, J=2 Hz), 12.02—12.39 (1H, br s). *Anal.* Calcd for $C_{11}H_{12}ClNO_3$: C, 54.67; H, 5.00; N, 5.80. Found: C, 54.57; H, 5.05; N, 5.80. MS *m/z*: 241 (M^+).

6-Chloro-3,4-dihydro-4-methyl-2-phenyl-2*H*-1,4-benzoxazine-8-carboxylic Acid (19b) Yield 76%, mp 165—166 °C (EtOH). ^{1}H-NMR (DMSO-d_6) δ: 2.81 (3H, s), 3.11—3.78 (2H, m), 5.14—5.32 (1H, m), 6.78 (1H, d, J=2 Hz), 6.83 (1H, d, J=2 Hz), 7.21—7.52 (5H, m), 12.44—12.78 (1H, br s). *Anal.* Calcd for $C_{16}H_{14}ClNO_3$: C, 63.27; H, 4.65; N, 4.61. Found: C, 62.98; H, 4.69; N, 4.58. MS *m/z*: 303 (M^+).

6-Chloro-3,4-dihydro-2,2,4-tetramethyl-2*H*-1,4-benzoxazine-8-carboxylic Acid (19c) Yield 86%, mp 169—170 °C (dec.). ^{1}H-NMR (DMSO-d_6) δ: 1.47 (6H, s), 2.95 (3H, s), 3.14 (2H, s), 6.79 (1H, d, J=2 Hz), 7.42 (1H, d, J=2 Hz), 10.71—10.93 (1H, br s). *Anal.* Calcd for $C_{12}H_{14}ClNO_3$: C, 56.37; H, 5.52; N, 5.48. Found: C, 56.33; H, 5.53; N, 5.52. MS *m/z*: 255 (M^+).

General Procedure for the Preparation of Benzamides (15a, 15b, 20a—c and 21—28) Physical and spectral data for **15a, 15b, 20a—c** and **21—28** are listed in Tables 1, 2 and 4. Pivaloyl chloride (1.2 ml, 10 mmol) was added to a mixture of the carboxylic acid **14a, 14b** or **19a—d** (10 mmol), NEt_3 (1.4 ml, 10 mmol), and AcOEt (30 ml) at 0 °C. The whole was stirred at below 5 °C for 30 min, and a solution of the appropriate amine (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 or converted to the hydrochloride in the usual manner.

NMR Studies of 27 and 28 The ^{1}H-NMR spectra of **27**·HCl and **28**·HCl (deuteriodimethyl sulfoxide) and the free bases of **27** and **28** (deuteriochloroform) were obtained at 400 MHz using spectral widths of 8000 Hz and acquisition times of 2.0 s. Measurements were made at

Table 4. ^{1}H-NMR Spectral Data for Compounds **15a, 15b, 20a—c**, and **21—26** (100 MHz)

15a	1.40—1.88 (4H, m), 1.98—2.12 (1H, m), 2.46—3.04 (5H, m), 2.89 (2H, t, J=5), 3.00 (3H, s), 3.23—3.60 (1H, m), 3.65
(DMSO-d_6)	(2H, t, J=5), 3.92—4.28 (1H, m), 5.90—6.21 (1H, br s), 6.65 (1H, d, J=2), 6.43 (1H, d, J=2)
15b	1.63—2.23 (7H, m), 2.86 (3H, s), 2.87—3.08 (3H, m), 3.10—3.29 (3H, m), 3.30—3.52 (1H, m), 3.55—3.68 (1H, m),
(DMSO-d_6)	3.70—3.88 (2H, m), 4.13—4.29 (1H, m), 6.81 (1H, d, J=2), 6.87 (1H, d, J=2), 8.63—8.74 (1H, br s), 10.51—10.70 (1H, br s)
20a	1.30 (3H, d, J=5), 1.57—2.04 (4H, m), 2.04—2.28 (1H, m), 2.87 (3H, s), 2.92—3.34 (7H, m), 3.38—3.78 (1H, m),
(DMSO-d_6)	4.08—4.49 (2H, m), 8.28—8.50 (1H, br s), 10.32—10.54 (1H, br s)
20b	0.90—1.24 (2H, m), 1.41—1.68 (2H, m), 1.72—1.88 (1H, m), 2.01—2.82 (5H, m), 2.96 (3H, s), 3.08—3.52 (3H, m),
($CDCl_3$)	3.96—4.21 (1H, m), 5.04—5.26 (1H, m), 6.73 (1H, d, J=3), 7.25—7.44 (5H, m), 7.48 (1H, d, J=3), 7.96—8.19 (1H, br s)
20c	1.29 (3H, s), 1.32 (3H, s), 1.61—2.08 (4H, m), 2.06—2.28 (1H, m), 2.92 (3H, s), 2.98—3.32 (5H, m), 3.36 (2H, s), 3.40—3.78
(DMSO-d_6)	(1H, m), 4.08—4.41 (1H, m), 6.79 (1H, d, J=1), 6.81 (1H, d, J=1), 8.22—8.44 (1H, br s), 10.68—10.92 (1H, br s)
21	1.24 (6H, t, J=7), 2.88 (3H, s), 3.08—3.24 (6H, m), 3.30 (2H, t, J=4), 3.57—3.70 (2H, m), 4.33 (2H, t, J=4), 6.77 (1H, d,
(DMSO-d_6)	J=2), 6.94 (1H, d, J=2), 8.52—8.63 (1H, br s), 10.41—10.57 (1H, br s)
22	2.90 (3H, s), 3.28 (4H, t, J=5), 3.01—3.62 (4H, m), 3.68 (2H, t, J=4), 3.90 (4H, t, J=5), 4.34 (2H, t, J=4), 6.77 (1H, d,
(DMSO-d_6)	J=3), 6.94 (1H, d, J=3), 8.44—8.68 (1H, br s), 11.24—11.35 (1H, br s)
23	1.38—1.87 (4H, m), 1.97—2.31 (1H, m), 2.30—2.78 (4H, m), 2.91 (3H, s), 3.37 (2H, t, J=4), 3.50 (2H, s), 4.42 (2H, t,
($CDCl_3$)	J=4), 6.69 (1H, d, J=3), 7.10—7.38 (5H, m), 7.43 (1H, d, J=3), 8.07—8.38 (1H, br s)
24	2.90 (3H, s), 3.08—3.49 (3H, m), 3.35 (2H, t, J=4), 3.84—4.13 (2H, m), 4.17—4.42 (2H, m), 4.32 (2H, t, J=4), 4.42—4.52
(DMSO-d_6)	(4H, m), 6.75 (1H, d, J=3), 6.83 (1H, d, J=3), 7.36—7.82 (5H, m), 8.16—8.41 (1H, br s), 11.41—11.87 (1H, br s)
25	1.85—2.32 (5H, m), 2.34—2.77 (3H, m), 2.89 (3H, s), 3.34 (2H, t, J=6), 3.63—4.06 (3H, m), 4.31 (2H, t, J=6), 6.76 (1H, d,
(DMSO-d_6)	J=2), 6.82 (1H, d, J=2), 8.21—8.40 (1H, br s), 10.42—10.73 (1H, br s, HCl)
26	1.22—1.92 (5H, m), 1.93—2.28 (3H, m), 2.66—2.82 (2H, m), 2.89 (3H, s), 3.28 (2H, t, J=6), 3.39—3.70 (3H, m), 4.28
(DMSO-d_6)	(2H, t, J=6), 6.76 (2H, s), 7.97—8.17 (1H, br s), 9.41—9.67 (1H, br s, HCl)

23 °C.

Compound **27**·HCl, mp 289 °C (dec.). ^{1}H-NMR (DMSO-d_6) δ: 1.32 (6H, s), 1.89 and 2.11 (2H, d, J=16.1, 15.6 Hz, respectively), 2.24, 2.35 (4H, m), 2.49 (2H, m), 2.64, 2.79 (3H, d, J=4.9, 4.9 Hz, respectively), 2.92 (3H, s), 3.11 (2H, s), 3.73, 3.84 (2H, m), 3.95, 4.02 (1H, m), 6.80 (1H, d, J=2.4 Hz), 6.86, 6.88 (1H, d, J=2.4, 2.4 Hz, respectively), 8.16, 8.19 (1H, br s), 10.56, 10.76 (1H, br s).

Compound **28**·HCl, mp 262 °C (dec.). ^{1}H-NMR (DMSO-d_6) δ: 1.31 (6H, s), 1.47, 1.69 (5H, m), 2.03, 2.22 (3H, m), 2.53 (2H, m), 2.79 (3H, s), 2.92 (3H, s), 3.09 (2H, s), 2.43, 3.57 (2H, m), 4.21, 4.51 (1H, m), 6.79 (1H, d, J=2.5 Hz), 6.85, 6.90 (1H, d, J=2.5, 2.5 Hz, respectively), 7.98, 8.23 (1H, br s), 9.79, 10.68 (1H, br s).

A solution of **27**·HCl (200 mg, 0.48 mmol) in water was alkalized with aqueous K_2CO_3, and extracted with $CHCl_3$ (10 ml × 3). The separated organic layer was dried over $MgSO_4$ and evaporated at reduced pressure to afford a white solid. The resulting solid was recrystallized from EtOH–IPE to give the free base of **27** (152 mg, 84%) as colorless crystals, mp 190—192 °C. ^{1}H-NMR (CD_3OD) δ: 1.36 (6H, s, 2C$\underline{H}_3$), 1.65 (2H, d, J=14.0 Hz, H22 and H42), 1.78 (2H, dd, J=15.1, 7.0 Hz, H62 and H72), 2.05 (2H, m, H71 and H61), 2.18 (2H, m, H21 and H41), 2.23 (3H, s, N–C$\underline{H}_3$), 2.87 (3H, s, N–C$\underline{H}_3$), 3.00 (2H, s, C$\underline{H}_2$), 3.08 (2H, m, H1 and H5), 4.17 (1H, t, J=7.1 Hz, H3), 6.62 (2H, d, J=2.4 Hz, Ar-H), 7.42 (2H, d, J=2.4 Hz, Ar-H). *Anal.* Calcd for $C_{20}H_{28}ClN_3O_2$: C, 63.56; H, 7.47; N, 11.12. Found: C, 63.37; H, 7.47; N, 10.95. MS m/z: 377 (M^+).

The free base of **28** was prepared from **28**·HCl by the same procedure as described for the free base of **27**. Yield 86%, pearl yellow crystals, mp 135—137 °C. ^{1}H-NMR (CD_3OD) δ: 1.00 (2H, d, J=9.8 Hz, H61 and H81), 1.17 (2H, ddd, J=13.7, 10.3, 2.9 Hz, H22 and H42), 1.33 (6H, s, 2C$\underline{H}_3$), 1.45 (1H, dd, J=18.1, 13.2 Hz, H72), 1.87 (3H, m, H62, H71 and H82), 2.42 (3H, s, N–C$\underline{H}_3$), 2.46 (2H, m, H21 and H41), 2.86 (3H, s, N–C$\underline{H}_3$), 2.97 (2H, m, H1 and H5), 2.99 (2H, s, C$\underline{H}_2$), 4.35 (1H, tt, J=10.5, 6.9 Hz, H3), 6.60 (2H, d, J=2.4 Hz, Ar-H), 7.42 (2H, d, J=2.4 Hz, Ar-H). *Anal.* Calcd for $C_{21}H_{30}ClN_3O_2$: C, 64.35; H, 7.71; N, 10.72. Found: C, 64.02; H, 7.78; N, 10.53. MS m/z: 391 (M^+).

General Procedure for the Enantiomers of *N*-(1-Azabicyclo[2.2.2]oct-3-yl)-3,4-dihydro-2*H*-1,4-benzoxazine-8-carboxamides (20c) Pivaloyl chloride (1.2 ml, 10 mmol) was added to a mixture of the carboxylic acid **19c** (2.3 g, 10 mmol), NEt_3 (1.4 ml, 10 mmol), and AcOEt (30 ml) at 0 °C. The whole 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 at room temperature for 1 h, then 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*)-**20c**. Physical data for (*S*)- or (*R*)-**20c** are listed in Table 2.

[^{3}H]Granisetron Binding [^{3}H]Granisetron binding assay was performed according to the method of Nelson and Thomas.[19] 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 mixture 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.[20] 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, Shimidzu) was injected intravenously. The test drug was administered 5 min before the rapid bolus injection of 5-HT (10 or 20 μg/kg). To assess inhibition of 5-HT-induced changes in heart rate, the statistical significance of differences between mean values was determined by using Student's test for paired data. The criterion of statistical significance was $p<0.05$. The ED_{50} value of each test drug was determined by a modification of the method of Waud[21] as the dose which suppressed the bradycardia by 50%.

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17) Crystal data of **28**: $C_{21}H_{30}ClN_3O_2 \cdot HCl \cdot H_2O$, M_r=482.5, triclinic, $P\bar{1}$, a=10.967(2), b=26.692(4), c=8.964(1) Å, α=93.29(3), β=101.93(2), γ=85.37(3)°, V=2556.8(8) Å^3, Z=4, D_{calcd}=1.253 g cm^{-3}. Intensities were collected on an Enraf-Nonius CAD-4 diffractometer with graphite-monochromated CuK_α radiation (λ=1.5418 Å). Unique 4144 reflections with $I>2.3\sigma(I)$ were used for the refinement. The structure was solved by the direct method. Atomic parameters were refined by a block-diagonal least-squares method and the final R value was 0.074.
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