Synthesis and Pharmacology of 3,4-Dihydro-3-oxo-1,4-benzoxazine-8-carboxamide Derivatives, a New Class of Potent Serotonin-3 (5-HT_3) Receptor Antagonists

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A series of 3,4-dihydro-3-oxo-1,4-benzoxazine-8-carboxamide derivatives was synthesized and evaluated for serotonin-3 (5-HT_3) receptor antagonistic activity assessed by their ability to antagonize the von Bezold-Jarish (BJ) effect in rats. Derivatives bearing 1-azabicyclo[2.2.2]oct-3-yl moiety as a basic function attached to the carboxamide at position 8 showed more potent antagonistic activity than those bearing the other three basic moieties. Structure–activity relationships of this series showed that methyl and chloro groups were more effective as substituents at positions 4 and 6, respectively. The representative compound 15 (Y-25130) in this series showed potent antagonistic activity on the BJ effect (ED_{50} = 1.3 μg/kg i.v.), high affinity for 5-HT_3 receptor (K_i = 2.9 nM) and complete protection against cisplatin-induced emesis in dogs at a dose of 0.1 mg/kg i.v.

Keywords 3,4-dihydro-3-oxo-1,4-benzoxazine-8-carboxamide derivative; benzamide analogue; serotonin-3 (5-HT_3) receptor antagonist; structure–activity relationship; von Bezold-Jarish effect; antiemetic; chemotherapy-induced emesis

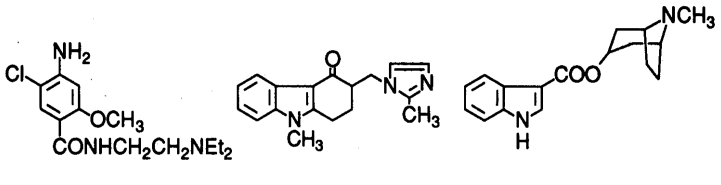
Introduction

Chemotherapeutic agents used in the treatment of cancer patients are well known to produce severe gastrointestinal side effects such as nausea and vomiting.[1] High intravenous doses of metoclopramide (**1**) have been used clinically in the management of nausea and vomiting.[2] However, **1** produces extrapyramidal side effects which are due to its dopamine receptor antagonistic activity.[3] Compound **1** is also a relatively weak serotonin-3 (5-HT_3) receptor antagonist, and its antiemetic activity at high dosage in clinical treatments has been explained by the antagonism of 5-HT_3 receptors.[4]

It has been reported that selective 5-HT_3 receptor antagonists including ondansetron (**2**)[5] and ICS 205,930 (**3**)[6] were effective as antiemetics in the treatment of the severe nausea and vomiting induced by chemotherapy in animals and patients.

Since the 5-HT_3 receptor is distributed not only in the peripheral tissues but also in the brain,[7] its antagonists are expected to play a useful role in the treatment of gastrointestinal motility disturbance, migraine, or central nervous system disorders.[8]

Our efforts in this study were focussed on searching for a new class of benzamide analogue with a potent 5-HT_3 antagonistic activity devoid of dopamine receptor antagonistic property. Benzamides with dopamine receptor



metoclopramide (1) ondansetron (2) ICS 205,930 (3)

Chart 1

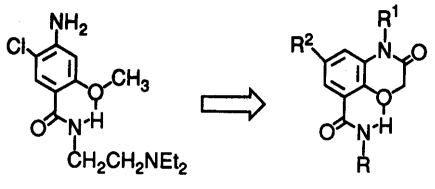


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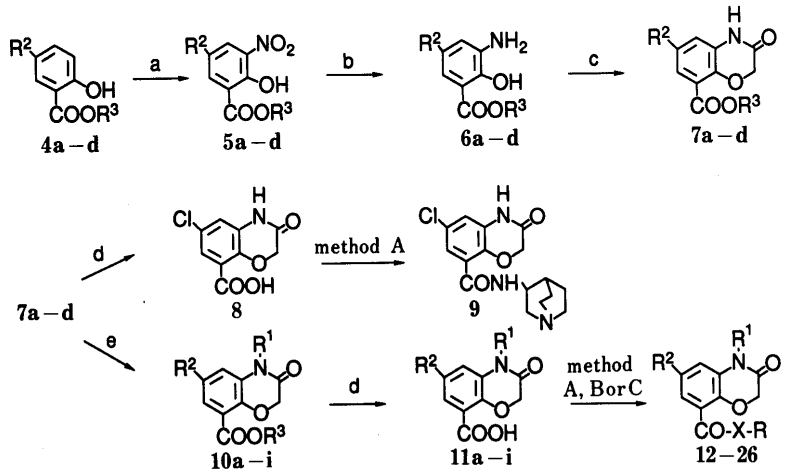
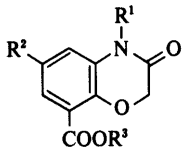
Chart 2



reagents: a)HNO_3, H_2SO_4, b)Fe powder, 0.78 N NH_4Cl, toluene, c)i: $ClCH_2COCl$, aq.$NaHCO_3$, $CHCl_3$, ii: K_2CO_3, d)NaOH, H_2O-EtOH, e)RX, *tert*-BuOK or K_2CO_3, method A i)EtOCOCl or pivaloyl chloride, NEt_3,THF-DMF, ii)RNH_2, method B i)$SOCl_2$, ii)RNH_2, method C i)$SOCl_2$, ii)ROH, BuLi, THF

Chart 3

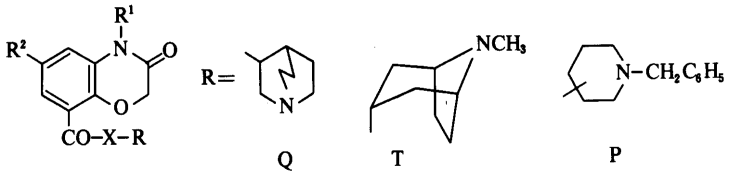
TABLE I. Physical Data for 3,4-Dihydro-3-oxo-1,4-benzoxazine Derivatives **7**, **8**, **10** and **11**



Compd. No.	R^1	R^2	R^3	Yield (%)	mp (°C) (Solvent)	Formula	Analysis (%) Calcd (Found)		
							C	H	N
7a	H	Cl	Me	90.3	239—241 (DMF–EtOH)	$C_{10}H_8ClNO_4$	49.41 (49.80	3.34 3.37	5.80 5.85)
7b	H	F	Et	70.0	189—190 (EtOH)	$C_{11}H_{10}FNO_4$	55.23 (55.29	4.21 4.27	5.86 5.86)
7c	H	Br	Me	81.0	256—257 (EtOH)	$C_{10}H_8BrNO_4$	41.99 (42.03	2.82 2.96	4.90 4.93)
7d	H	Me	Me	90.9	209—210 (EtOH)	$C_{11}H_{11}NO_4$	59.72 (59.39	5.01 4.94	6.33 6.46)
10a	Me	Cl	Me	82.2	150—152 (EtOH)	$C_{11}H_{10}ClNO_4$	51.68 (51.40	3.94 3.93	5.48 5.50)
10b	Me	F	Et	69.0	113—114 (EtOH)	$C_{12}H_{12}FNO_4$	56.92 (56.77	4.78 4.84	5.53 5.46)
10c	Me	Br	Me	54.0	154—155 (EtOH)	$C_{11}H_{10}BrNO_4$	44.02 (44.02	3.36 3.36	4.67 4.67)
10d	Me	Me	Me	79.1	110—112 (EtOH)	$C_{12}H_{13}NO_4$	61.27 (61.56	5.57 5.63	5.96 5.99)
10e	Et	Cl	Me	73.0	123—125 (EtOH)	$C_{12}H_{12}ClNO_4$	53.45 (53.48	4.49 4.57	5.19 5.15)
10f	Pr	Cl	Me	77.0	112—114 (EtOH)	$C_{13}H_{14}ClNO_4$	55.04 (55.30	4.97 5.08	4.94 4.99)
10g	Bu	Cl	Me	79.0	73—74 (EtOH)	$C_{14}H_{16}ClNO_4$	56.48 (56.38	5.42 5.48	4.70 4.67)
10h	$CH_2C_6H_5$	Cl	Me	72.0	109—111 (EtOH)	$C_{17}H_{14}ClNO_4$	61.55 (61.40	4.25 4.40	4.22 4.31)
10i	$CH_2CH_2C_6H_5$	Cl	Me	64.0	84—86 (EtOH)	$C_{18}H_{16}ClNO_4$	62.52 (62.52	4.66 4.76	4.05 3.99)
8	H	Cl	H	86.2	323—324 (DMF–EtOH)	$C_9H_6ClNO_4$	47.49 (47.65	2.66 2.69	6.15 6.29)
11a	Me	Cl	H	81.5	236—238 (DMF–EtOH)	$C_{10}H_8ClNO_4$	49.71 (49.67	3.34 3.32	5.80 5.86)
11b	Me	F	H	77.0	164—166 (DMF–EtOH)	$C_{10}H_8FNO_4$	53.34 (53.21	3.58 3.71	6.22 6.11)
11c	Me	Br	H	96.7	192—194 (DMF–EtOH)	$C_{10}H_8BrNO_4$	41.99 (42.08	2.82 2.93	4.90 4.88)
11d	Me	Me	H	98.3	192—194 (EtOH)	$C_{11}H_{11}NO_4 \cdot H_2O$	55.23 (55.40	5.48 5.34	5.87 5.93)
11e	Et	Cl	H	76.6	148—150 (DMF–EtOH)	$C_{11}H_{10}ClNO_4$	51.68 (51.66	3.94 4.06	5.48 5.60)
11f	Pr	Cl	H	88.0	169—171 (EtOH)	$C_{12}H_{12}ClNO_4$	53.45 (53.34	4.49 4.58	5.19 5.19)
11g	Bu	Cl	H	70.3	153—154 (EtOH)	$C_{13}H_{14}ClNO_4$	55.04 (55.24	4.97 5.11	4.94 4.80)
11h	$CH_2C_6H_5$	Cl	H	79.0	143—145 (EtOH)	$C_{16}H_{12}ClNO_4$	60.48 (60.32	3.81 3.82	4.41 4.39)
11i	$CH_2CH_2C_6H_5$	Cl	H	90.0	152—153 (EtOH)	$C_{17}H_{14}ClNO_4$	61.55 (61.57	4.25 4.38	4.22 4.15)
11j	Me	H	H	97.5	216—217 (EtOH)	$C_{10}H_9NO_4$	57.97 (57.94	4.38 4.40	6.76 6.70)
11k	Me	NO_2	H	76.2	270 (dec.) (DMF–EtOH)	$C_{10}H_8N_2O_6$	47.63 (47.50	3.20 3.34	11.11 11.30)

antagonistic activity, such as **1**, sulpiride and remoxipride, have a common structural feature,[9] which is an intramolecular hydrogen bonding between the amide moiety and the *o*-methoxy oxygen atom. As shown in Chart 2, the hydrogen bonding in **1** has been thought to stabilize the active conformation in which the phenyl ring is coplanar to the carbonyl group oriented in the direction opposite that of the methoxy group side, and this stabilized conformation may contribute to enhancement of the pharmacological activity. In that case, it is expected that the activity will be more enhanced by strengthening or stabilizing the hydrogen bond. The hydrogen bond in the benzamide is interfered with by the rotation of C–O bond between the phenyl ring and the methoxy group. When the *O*-alkyl substituent is devised to make an appropriate linkage to the *m*-position of the benzamide, the rotation

TABLE II. Structure and 5-HT_3 Receptor Antagonistic Activity of 3,4-Dihydro-3-oxo-1,4-benzoxazines **9**, **12**—**30**



Q T P

Compd. No.	R^1	R^2	X	R	Yield (%) Method[a]	mp (°C) (Solvent)	Formula	Analysis (%) Calcd (Found) C	H	N	Anti-BJ effect[f] ED_{50} (μg/kg i.v.)
9	H	Cl	NH	Q	19.3 A	223 (dec.) (EtOH)	$C_{16}H_{18}ClN_3O_3 \cdot HCl \cdot 2/3H_2O$	48.13 (47.83)	5.55 5.13	10.52 10.51)	12.9 (10.7—15.8)[g]
12	Me	Cl	NH	$CH_2CH_2NEt_2$	44.0 A	140—142 (EtOH)	$C_{16}H_{22}ClN_3O_3 \cdot HCl \cdot 1/4H_2O$	50.47 (50.25)	6.22 6.17	11.04 10.95)	>1000
13	Me	Cl	NH	3-P[b]	24.3 A	218—219 (EtOH–AcOEt)	$C_{22}H_{24}ClN_3O_3 \cdot HCl$	58.61 (58.41)	5.55 5.70	9.32 9.16)	>1000
14	Me	Cl	NH	4-P[c]	57.4 A	233—235 (EtOH–H_2O)	$C_{22}H_{24}ClN_3O_3 \cdot C_2H_2O_4$	57.15 (57.39)	5.16 5.29	8.33 8.28)	>1000
15	Me	Cl	NH	Q	82.9 B	305 (dec.) (EtOH)	$C_{17}H_{20}ClN_3O_3 \cdot HCl$	52.86 (52.77)	5.48 5.47	10.88 10.79)	1.3 (0.9—2.0)
16	Me	Cl	NH	T	31.1 B	308 (dec.) (EtOH–IPE)[d]	$C_{18}H_{22}ClN_3O_3 \cdot HCl \cdot 1/2H_2O$	52.82 (52.64)	5.91 6.19	10.27 9.97)	1.9 (1.2—3.0)
17	Me	Cl	O	Q	19.8 C	295 (dec.) (MeOH)	$C_{17}H_{19}ClN_2O_4 \cdot HCl$	52.73 (52.11)	5.21 5.16	7.23 7.18)	5.9 (4.7—7.3)
18	Me	Cl	O	T	12.7 C	127 (dec.) (EtOH)	$C_{18}H_{21}ClN_2O_4 \cdot HCl \cdot 3/4H_2O$	52.12 (52.14)	5.71 5.57	6.75 6.70)	4.7 (2.9—7.5)
19	Et	Cl	NH	Q	55.5 A	304 (dec.) (MeOH)	$C_{18}H_{22}ClN_3O_3 \cdot HCl$	54.01 (53.99)	5.79 5.87	10.50 10.34)	8.6 (5.5—12.9)
20	Pr	Cl	NH	Q	74.7 A	250—252 (EtOH)	$C_{19}H_{24}ClN_3O_3 \cdot HCl \cdot 1/4H_2O$	54.49 (54.68)	6.14 6.03	10.03 10.03)	13.0 (8.5—19.6)
21	Bu	Cl	NH	Q	51.0 A	241—243 (EtOH)	$C_{20}H_{26}ClN_3O_3 \cdot HCl \cdot 1/4H_2O$	55.50 (55.45)	6.40 6.30	9.71 9.60)	46.8 (30.2—72.0)
22	$CH_2C_6H_5$	Cl	NH	Q	59.0 A	283—285 (EtOH)	$C_{23}H_{24}ClN_3O_3 \cdot HCl \cdot 1/4H_2O$	59.17 (59.21	5.51 5.51	9.00 8.91)	35.8 (26.0—47.4)
23	$CH_2CH_2C_6H_5$	Cl	NH	Q	29.0 A	247—249 (EtOH)	$C_{24}H_{26}ClN_3O_3 \cdot HCl \cdot 1/4H_2O$	59.94 (59.80	5.76 6.02	8.74 8.57)	12.5 (8.7—17.7)
24	Me	F	NH	Q	66.4 B	286 (dec.) (EtOH)	$C_{17}H_{20}FN_3O_3 \cdot HCl$	55.16 (55.05	5.68 5.72	11.36 11.13)	5.8 (4.2—7.9)
25	Me	Br	NH	Q	45.0 B	>300 (EtOH)	$C_{17}H_{20}BrN_3O_3 \cdot HCl \cdot 1/4H_2O$	46.86 (46.68	4.94 4.94	9.65 9.65)	4.0 (3.1—5.3)
26	Me	Me	NH	Q	55.4 B	284 (dec.) (EtOH)	$C_{18}H_{23}N_3O_3 \cdot HCl$	59.09 (58.73	6.61 6.65	11.49 11.38)	3.8 (3.1—4.7)
27	Me	H	NH	Q	61.5 B	272 (dec.) (MeOH–IPE)	$C_{17}H_{21}N_3O_3 \cdot HCl$	58.03 (58.23	6.30 6.26	11.94 11.78)	5.9 (4.9—7.3)
28	Me	NO_2	NH	Q	45.5 B	307 (dec.) (MeOH)	$C_{17}H_{20}N_4O_5 \cdot HCl$	51.45 (51.17	5.33 5.31	14.12 14.01)	310 (180—184)
29	Me	NH_2	NH	Q	70.3	271 (dec.) (EtOH)	$C_{17}H_{22}N_4O_3 \cdot 2HCl \cdot 1/2H_2O$	49.52 (49.79	6.11 6.02	13.59 13.72)	1.7 (1.1—2.7)
30	Me	OH	NH	Q	7.6	250—251 (IPA)[e]	$C_{17}H_{21}N_3O_4$	61.62 (61.43	6.39 6.40	12.68 12.51)	1.4 (0.9—2.3)
1	Metoclopramide										1161 (866—1538)
2	Ondansetron										5.7 (1.5—3.3)
3	ICS 205, 930										2.3 (1.5—3.3)

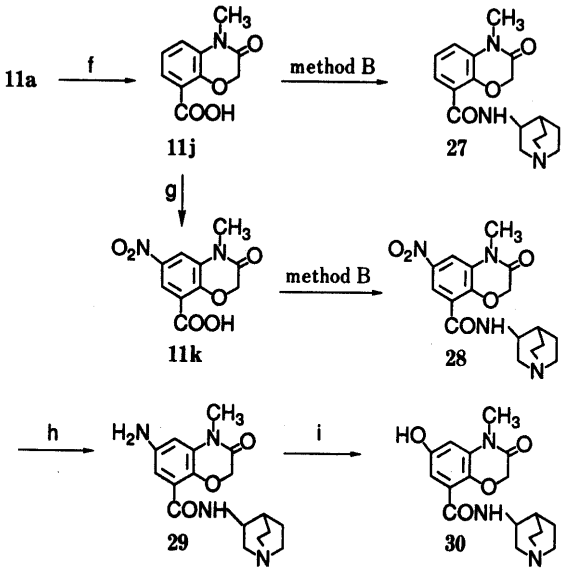
a) See the Experimental section. *b*) 1-Benzyl-3-piperidinyl. *c*) 1-Benzyl-4-piperidinyl. *d*) Isopropyl ether. *e*) Isopropanol. *f*) Antagonism of von Benzold-Jarish effect induced by i.v. injection of 20 μg/kg 5-HT. *g*) 95% confidence limits.

of C–O bond is fixed and the formation of the hydrogen bond will not be interrupted.[10]

This concept led us to synthesize 3,4-dihydro-3-oxo-1,4-benzoxazine-8-carboxamide derivatives (Chart 2). The selectivity for 5-HT_3 receptor may be achieved by the use of bulky substituents in place of the conformationally free diethylaminoethyl side chain of **1**.[11]

In the present paper, we describe the synthesis and 5-HT_3 receptor antagonistic activities of 3,4-dihydro-3-oxo-1,4-benzoxazine-8-carboxamides and the antiemetic activities of the representative compound in this series.

Chemistry Compounds **9** and **12**—**26** were prepared as shown in Chart 3. The salicylates (**4a**—**d**) were nitrated with conc. HNO_3 and conc. H_2SO_4 followed by reduction of the nitro group with Fe powder under the neutral condition to afford **6a**—**d**. Compounds **6a**—**d** were acylated



reagents: f)H_2,Pd/C,EtOH, g)HNO_3, H_2SO_4, h)H_2,Raney Ni,EtOH, i)i:$NaNO_2$,dil. H_2SO_4, ii:CuO,$Cu(NO_2)_2$, method B i)$SOCl_2$, ii)RNH_2

Chart 4

with chloroacetyl chloride followed by cyclization in the presence of K_2CO_3 to afford **7a—d**.

Compounds **7a—d** were *N*-alkylated with appropriate alkyl halides in the presence of base to afford **10a—i**. The carboxylic acids **8** and **11a—i** were obtained by hydrolysis of the corresponding esters **7a** and **10a—i**, respectively.

The amides listed in Table II were prepared from the corresponding carboxylic acids by coupling reaction with appropriate amines *via* the mixed anhydrides (method A) or the acid chlorides (method B). The esters **17** and **18** were prepared by the reaction of the corresponding acid chlorides with lithium alcoholates in tetrahydrofuran (THF, method C).[12]

Compound **11j** was obtained by reductive dechlorination of **11a** in the presence of Pd catalyst. Compound **27** was prepared from **11j** and 3-amino-1-azabicyclo[2.2.2]octane *via* the mixed anhydride. Compound **28** was prepared by nitration of **11j** followed by condensation with the amine. The 6-amino compound **29** obtained by reduction of **28** was converted to the 6-hydroxy compound **30** according to the procedure of Cohen.[13] Amines (3-amino-1-azabicyclo-[2.2.2]octane and *endo*-3-amino-8-methyl-8-azabicyclo-[3.2.1]octane) were prepared from the corresponding 3-oxo derivatives *via* their oximes according to the reported procedure.[14]

Pharmacological Results and Discussion

The synthesized compounds **9**, **12—30** (Table II) and **1—3** were tested for the 5-HT_3 receptor antagonistic activity, which was evaluated by their ability to antagonize the 5-HT induced bradycardia (von Bezold-Jarish (BJ) effect) in rats. The BJ effect is the result of reflex stimulation of the vagus nerve following activation of 5-HT_3 receptors located in the wall of the right ventricle.[15]

Compound **12** bearing the diethylaminoethyl moiety attached to the nitrogen atom of the carboxamide at position 8 as the basic side chain did not show any antagonistic activity for the BJ effect at the dose of up to 1000 μg/kg i.v., nor did compounds bearing 1-benzylpiperidinyl moiety (**13** and **14**). However, introduction of more sterically bulky amines (1-azabicyclo[2.2.2]oct-3-yl and 8-methyl-8-azabicyclo[3.2.1]oct-3-yl) as the basic moiety produced remarkable increase of the activity as seen in the ED_{50} values obtained for **15** and **16**. Compound **15** bearing 1-azabicyclo[2.2.2]oct-3-yl moiety, in particular, was more potent than **16** and the reference compounds **2** and **3**.

TABLE III. Binding Affinities and Antiemetic Activities of **15** and **1**

Test	Index	Animals	Compounds	
			15	**1**
Binding affinity				
[^{3}H]Quipazine	K_i (M)	Rat cortex	2.9×10^{-9}	8.7×10^{-7}
[^{3}H]Spiperone	K_i (M)	Rat striatal	$>10^{-5}$ [a]	1.0×10^{-7}
Antiemesis				
Cisplatin (3 mg/kg i.v.)	ED_{100} [b] (mg/kg i.v.)	Dogs	0.1 (0/9) [c]	>4 (3/5) [d]
Cyclophosphamide/ doxorubucin (80 mg/kg–6 mg/kg i.v.)	ED_{100} [b] (mg/kg i.v.)	Ferrets	0.3 (0/6)	>4 (5/6)

a) IC_{50} value. *b*) ED_{100} is the dose which shows complete protection against the emesis. *c*) Number of dogs vomiting/tested. *d*) Number of ferrets vomiting/tested.

Compounds **17** and **18** bearing an ester moiety instead of the carboxamide at position 8 showed less potent activity compared with the corresponding carboxamide derivatives (**15** and **16**, respectively). The intramolecular hydrogen bonding between the amide moiety and the oxygen atom at the *ortho* position plays an important role in stabilizing the active conformation of benzamides analogue as mentioned above, but the ester compounds lack the ability to form the hydrogen bond. Indeed, the existence of the intramolecular hydrogen bond in the benzoxazine-amide molecule was confirmed by single-crystal X-ray diffraction analysis of **15**.[16] The lower activity of the ester compounds may be due to the absence of the hydrogen bonding, and/or to be subject to hydrolysis of the ester in the *in vivo* model such as BJ effect.

Therefore, we selected the *N*-(1-azabicyclo[2.2.2]oct-3-yl carboxamide as the basic moiety at position 8 and then investigated the influence of the substituents at positions 4 and 6 on the BJ effect.

The demethylated compound **9** at position 4 was about 10 times less potent than **15**. Replacement of methyl group by larger substituents resulted in reducing the activity in the order of Et (**19**)> $CH_2CH_2C_6H_5$ (**23**)> Pr (**20**)> $CH_2C_6H_5$ (**22**)> Bu (**21**). The fact that the methyl group is most suitable as substituents at position 4 may suggest a steric constraint against the 5-HT_3 receptor.

The 6-amino and 6-hydroxy compounds (**29** and **30**) showed comparable or slightly less activity compared to 6-chloro compound **15**. Replacement of chloro group at position 6 by fluoro, bromo, methyl, hydro or nitro group (**24—28**, respectively) resulted in reduced activity, although all compounds except **28** were well tolerated. Compound **28** was virtually inactive.

On the basis of these structure–activity relationships, **15** was selected for further evaluation, such as its affinity for 5-HT_3 and dopamine D_2 receptors and its antagonism against chemotherapy-induced emesis (Table III). The affinities of **15** and **1** for 5-HT_3 receptors were evaluated

using specific [^{3}H]quipazine binding to rat cerebral cortex membrane.

Compound **15** displayed high affinity for the [^{3}H]-quipazine binding site (K_i=2.9 nM), but **1** showed only moderate affinity as presumed from the value of the anti-BJ effect. In order to predict the incidence of extrapyramidal side effects, these compounds were tested for D_2 receptor binding affinity using [^{3}H]spiperone as a ligand. Compound **1** showed the same order of affinity to D_2 receptors as to 5-HT_3 receptors. However, **15** did not show any affinity to D_2 receptors up to a concentration of 10^{-5} M, and therefore it is unlikely that **15** can cause the incidence of extrapyramidal side effects.

Compound **15** inhibited cisplatin-induced emesis in dogs in a dose dependent manner ranging from 0.003 to 0.1 mg/kg. i.v.,[17] and showed complete protection at the dose of 0.1 mg/kg. It also completely inhibited the emesis induced by the combination treatment of doxorubcin and cyclophosphamide in ferrets at the dose of 0.3 mg/kg i.v. On the contrary, **1** did not show complete protection against either emetic models even at the high dose of 4 mg/kg i.v.

In conclusion, new potent 5-HT_3 receptor antagonists have been synthesized in the 3,4-dihydro-3-oxo-1,4-benzoxazine series. Compound **15** (Y-25130) showed especially potent antagonistic activity on the BJ effect, high affinity for 5-HT_3 receptors and antiemetic activity against chemotherapy-induced emesis. Compound **15** is a promising candidate and is under further investigation to assess its clinical usefulness.

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 are expressed in ppm, with tetramethyl silane as the internal standard. Infrared (IR) spectra were recorded on a JASCO IR-810 instrument. Low-resolution mass spectra (MS) were obtained by a JMS-O1SG spectrometer. Elemental analysis and measurement of these spectra were performed by Yoshitomi Pharmaceutical Industries, Ltd., Fukuoka, Japan. Ondansetron[18] and ICS 205,930[19] were prepared by the reported methods in our laboratory.

Methyl 5-Chloro-2-hydroxy-3-nitrobenzoate (5a) To a solution of **4a** (74.6 g, 0.4 mol) in conc. H_2SO_4 (200 ml) kept below 0 °C was added a mixture of HNO_3 (*d* 1.5, 27.7 g, 0.44 mol) and conc. H_2SO_4 (18.5 ml) with stirring at below 5 °C, and stirring continued 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 79.6 g (86%) of **5a** as pale yellow leaflets, mp 164—164 °C. IR (KBr) cm^{-1}: 3080, 1677. NMR (CD_3COOD) δ: 4.40 (3H, s, CH_3), 8.07, 8.14 (1H×2, each d, J=3 Hz, aromatic-H). MS *m/z*: 231 (M^+). *Anal.* Calcd for $C_8H_6ClNO_5$: C, 41.49; H, 2.61; N, 6.05. Found: C, 41.55; H, 2.56; N, 6.03.

Ethyl 5-Fluoro-2-hydroxy-3-nitrobenzoate (5b) Yield 92.0%. mp 77—79 °C. IR (KBr) cm^{-1}: 1690. NMR ($CDCl_3$) δ: 2.44 (3H, t, J=7 Hz, CH_3), 4.48 (2H, q, J=7 Hz, CH_2), 7.8—8.0 (2H, m, aromatic-H), 11.8 (1H, s, OH). MS *m/z*: 229 (M^+). *Anal.* Calcd for $C_9H_8FNO_5$: C, 47.17; H, 3.52; N, 6.11. Found: C, 47.23; H, 3.61; N, 6.09.

Methyl 5-Bromo-2-hydroxy-3-nitrobenzoate (5c) Yield 82.3%. mp 142—143 °C (from EtOH). IR (KBr) cm^{-1}: 1680. NMR ($CDCl_3$) δ: 4.04 (3H, s, CH_3), 8.22, 8.26 (1H×2, each d, J=0.3 Hz, aromatic-H). MS *m/z*: 310 (M^+). *Anal.* Calcd for $C_8H_6BrNO_5$: C, 34.81; H, 2.19; N, 5.07. Found: C, 34.72; H, 2.20; N, 4.99.

Methyl 2-Hydroxy-5-methyl-3-nitrobenzoate (5d) Yield 85.3%. mp 149—150 °C (from EtOH). IR (KBr) cm^{-1}: 1680. NMR ($CDCl_3$) δ: 2.36 (3H, s, CH_3), 4.00 (3H, s, CH_3), 7.9—8.0 (2H, m, aromatic-H), 11.64 (1H, s, OH). MS *m/z*: 211 (M^+). *Anal.* Calcd for $C_9H_9NO_5$: C, 51.19; H, 4.30; N, 6.63. Found: C, 51.38; N, 4.29; N, 6.67.

Methyl 3-Amino-5-chloro-2-hydroxybenzoate (6a) To an aqueous 0.78 N NH_4Cl solution (100 ml) was added Fe powder (33.4 g, 0.64 mol) with stirring at 85 °C followed by addition of a solution of **5a** (49.1 g, 0.21 mol) in toluene (500 ml) over 30 min. The reaction temperature rose to 95 °C. The reaction mixture was stirred at 85—90 °C for an additional 1 h, then filtered with suction through celite. The filtrate was poured into ice-water and the organic layer was separated, dried over $MgSO_4$ and evaporated to dryness. The residue was recrystallized from EtOH to afford 33.4 g (79%) of **6a** as pale yellow crystals, mp 92—93 °C. IR (KBr) cm^{-1}: 3440, 3320, 1680. NMR ($CDCl_3$) δ: 3.87 (2H, s, NH_2), 3.94 (3H, s, CH_3), 7.78, 8.17 (1H×2, each d, J=3 Hz, aromatic-H), 11.84 (1H, s, OH). MS *m/z*: 201 (M^+). *Anal.* Calcd for $C_8H_8ClNO_3$: C, 47.66; H, 4.00; N, 6.95. Found: C, 47.70; H, 3.98; N, 6.98.

Ethyl 3-Amino-5-fluoro-2-hydroxybenzoate (6b) Yield 85%. mp 56—58 °C. IR (KBr) cm^{-1}: 3450, 1680. NMR ($CDCl_3$) δ: 1.40 (3H, t, J=7 Hz, CH_3), 3.7—4.1 (2H, br s, NH_2), 4.38 (2H, q, J=7 Hz, CH_2), 6.58, 6.87 (1H×2, each dd, J=3, 8 Hz, aromatic-H), 10.2—10.6 (1H, br s, OH). MS *m/z*: 199 (M^+). *Anal.* Calcd for $C_9H_{10}FNO_3$: C, 54.27; H, 5.06; N, 7.03. Found: C, 54.55; H, 5.02; N, 6.88.

Methyl 3-Amino-5-bromo-2-hydroxybenzoate (6c) Yield 91.3%. mp 80—82 °C. IR (KBr) cm^{-1}: 3450, 1680. NMR ($CDCl_3$) δ: 3.93 (3H, s, CH_3), 3.8—4.3 (2H, br s, NH_2), 6.96, 7.32 (1H×2, each d, J=3 Hz, aromatic-H). MS *m/z*: 246 (M^+). *Anal.* Calcd for $C_8H_8BrNO_3$: C, 39.05; H, 3.28; N, 5.70. Found: C, 39.23; H, 3.22; N, 5.52.

Methyl 3-Amino-2-hydroxy-5-methylbenzoate (6d) Yield 69.0%. mp 74—75 °C (from EtOH). IR (KBr) cm^{-1}: 1695, 1675. NMR ($CDCl_3$) δ: 1.70 (3H, s, CH_3), 3.40 (3H, s, CH_3), 7.28, 7.90 (1H×2, each d, J=0.3 Hz, aromatic-H). MS *m/z*: 181 (M^+). *Anal.* Calcd for $C_9H_{11}NO_3$: C, 59.66; H, 6.12; N, 7.73. Found: C, 59.49; H, 6.14; N, 7.73.

Methyl 6-Chloro-3,4-dihydro-3-oxo-2*H*-1,4-benzoxazine-8-carboxylate (7a) To a mixture of **6a** (8.9 g, 44 mmol) in $CHCl_3$ (200 ml) and aqueous saturated $NaHCO_3$ (100 ml) was added portionwise chloroacetyl chloride (6.0 g, 53 mmol) with vigorous stirring at 0—10 °C and stirring continued for an additional 2 h. The separated organic layer was washed with water, dried over $MgSO_4$ and concentrated. To a solution of the residue (methyl 5-chloro-3-(chloroacetamido)-2-hydroxybenzoate) in dimethylformamide (DMF, 70 ml) was added K_2CO_3 and this was stirred at 70 °C for 2 h. After cooling, the mixture was poured into water, then extracted with $CHCl_3$. The extract was washed with water, dried over $MgSO_4$ and concentrated to dryness. The residue was recrystallized from EtOH to afford 9.6 g (90.3%) of **7a** as white needles. Compounds **7b—d** were prepared similarly from **6b—d**. The physical data are listed in Table I.

Methyl 6-Chloro-3,4-dihydro-4-methyl-3-oxo-2*H*-1,4-benzoxazine-8-carboxylate (10a) To a mixture of **7a** (9.7 g, 40 mmol), DMF (50 ml) and K_2CO_3 (8.3 g, 60 mmol) was added methyl iodide (7.4 g, 52 mmol) with stirring at 10—18 °C and stirring continued at room temperature for 3 h, then at 40—45 °C for an additional 1 h. The reaction mixture was poured into cold water, and the precipitates were collected and washed with water. Recrystallization from EtOH afforded 8.4 g (82.2%) of **10a** as white crystals. Compounds **10b—i** were prepared similarly. The physical data are listed in Table I.

6-Chloro-3,4-dihydro-3-oxo-2*H*-1,4-benzoxazine-8-carboxylic Acid (8) A mixture of **7a** (4.6 g, 19 mmol), EtOH (20 ml) and 5% NaOH (60 ml) was refluxed for 3 h. The reaction mixture was acidified with 10% HCl at below 10 °C and the precipitates were collected and washed with cold water. Recrystallization from DMF–EtOH afforded 3.73 g (86.2%) of **8** as white needles. Compounds **11a—i** were prepared similarly. The physical data are listed in Table I.

General Procedure for 3,4-Dihydro-3-oxo-1,4-benzoxazine-8-carboxamide and Carboxylate Derivatives (Table II) Method A: To a mixture of **8** or **11** (10 mmol), NEt_3 (10 mmol), DMF (10 ml) and THF (30 ml) was added ethyl chloroformate or pivaloyl chloride (10 mmol) at −20 °C. The mixture was stirred at below −10 °C for 30 min, and a solution of the appropriate amine (10 mmol) in THF (5 ml) was added with stirring at −20 °C. After stirring the mixture at −10 °C for 30 min, and then at room temperature for 1 h, water was added and extracted with AcOEt. The extract was washed with water, dried over $MgSO_4$ and evaporated to dryness. The residue was chromatographed on silica gel, recrystallized and converted to the hydrochloride in the usual manner.

Method B: A mixture of the carboxylic acid (10 mmol), thionyl chloride (12 mmol), DMF (1 drop) and 1,2-dichloroethane (20 ml) was heated at 60 °C for 2 h. After cooling, the precipitates (acid chloride) were collected and washed with a small amount of 1,2-dichloroethane for use in the next procedure without further purification. A solution of the acid chloride in $CHCl_3$ (or CH_3CN) (10 ml) was added to a solution

of 3-amino-1-azabicyclo[2.2.2]octane (10 mmol) in $CHCl_3$ (or CH_3CN) (30 ml) at below 10 °C and stirred at room temperature for 1 h. The reaction mixture was made alkaline with aqueous Na_2CO_3. The separated organic layer was washed with water and dried over $MgSO_4$. After removal of the solvent, the residue was recrystallized from EtOH and converted to the hydrochloride in the usual manner.

Method C: Compounds **17** and **18** were prepared from the acid chloride of **11a** according to the procedure of Richardson *et al.*[19]

3,4-Dihydro-4-methyl-3-oxo-2*H*-1,4-benzoxazine-8-carboxylic Acid (11j) A mixture of **11a** (10 g, 41 mmol), NaOH (3.3 g), water (100 ml), EtOH (30 ml) and Pd–C (1.5 g) was stirred in a hydrogen atmosphere for 5.5 h. The reaction mixture was filtered and washed with water and the filtrate was acidified with 10% HCl. The precipitates were collected, washed with water to afford 8.3 g (97.8%) of crude **11j** which was recrystallized from EtOH. The physical data is listed in Table I.

6-Amino-*N*-(1-azabicyclo[2.2.2]oct-3-yl)-3,4-dihydro-4-methyl-3-oxo-2*H*-1,4-benzoxazine-8-carboxamide Hydrochloride (29) A mixture of **28** (14.2 g, 39.4 mmol), Raney Ni (3 g) and EtOH (250 ml) in a 500 ml autoclave was heated at 80 °C for 2 h. After cooling, the reaction mixture was filtered by suction through celite and washed with EtOH. The filtrate was concentrated to 50 ml and acidified with EtOH–HCl to afford the precipitates, which were collected, washed with EtOH and recrystallized from EtOH to afford 11.3 g (70.3%) of **29**.

***N*-(1-Azabicyclo[2.2.2]oct-3-yl)-3,4-dihydro-6-hydroxy-4-methyl-3-oxo-2*H*-1,4-benzoxazine-8-carboxamide Hydrochloride (30)** Compound **30** was prepared from **29** according to the procedure of Cohen[13] and its isolation was done as follows: The reaction mixture was adjusted to pH 10 with aqueous 48% NaOH, and $CHCl_3$ was added. The mixture was stirred vigorously and filtered by suction through celite. The separated organic layer was washed with water, dried over $MgSO_4$ and concentrated. The residue was chromatographed on silica gel eluting with $CHCl_3$–MeOH (10 : 1) and recrystallized from iso-PrOH to afford 0.86 g (7.6%) of **30**.

von Bezold-Jarish Effect The antagonism of 5-HT induced bradycardia was evaluated according to the methods 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. The amplified input from the transducer was used to drive a heart rate tachometer (San-ei, model 1321). Records were displayed on a rectigraph (San-ei, model 6174A). The jugular vein was cannulated for intravenous injections of the test drug and 5-HT. After completion of operative procedures, 100 units of heparine (Heparine sodium injection-N, Shimadzu) was injected intravenously. The test drug was administered 5 min before the rapid bolus injection of 5-HT, 20 μg/kg. The ED_{50} value of the test drug inhibiting the bradycardia by 50% was determined by a modification of the method of Waud.[21]

[^{3}H]Quipazine Binding to 5-HT$_3$ Receptors [^{3}H]Quipazine binding assays were performed according to the methods of Peroutka and Hamik.[22] Briefly, rat cerebral cortex was homogenized in 20 volumes of 0.32 M sucrose and centrifuged at 35000 × *g* for 15 min. The supernatant was discarded and the pellet resuspended in the same volume of Krebs–*N*-hydroxyethylpiperazine-*N*′-2-ethansulfonate (HEPES) buffer. After a 10 min incubation at 37 °C, the tissue was centrifuged for a second time. The final pellet was resuspended in 80 volumes of Krebs–HEPES buffer. The binding assay consisted of 50 μl [^{3}H]quipazine (Dupont New England Nuclear, Boston, MA), 50 μl displacing drug and 900 μl tissue homogenate. Following a 30 min incubation at 30 °C, the assay was rapidly filtered under vacuum through Whatman GF/B glass filters which had been presoaked in 0.1% polyethyleneimine. Filters were washed through with 3 × 3 ml of 50 mM Tris–HCl buffer (pH 7.7). ICS 205,930 (100 μM) was used for the determination of nonspecific binding. Radioactivity was measured by liquid scintillation spectrometry. IC_{50} values were determined from concentration–inhibition curves. K_i values were determined by the relationship $K_i = IC_{50}/(1 + c/k_d)$, where c is concentration of [^{3}H]-ligand and k_d is the dissociation constant of [^{3}H]-ligand.

[^{3}H]Spiperone Binding to Dopamine D$_2$ Receptors [^{3}H]Spiperone binding assays were performed according to the methods of Grees *et al.*[23] Briefly, rat striatal membranes were homogenized in 100 volumes of ice-cold Tris–HCl buffer (50 mM, pH 7.7) and centrifuged (500 × *g*, 10 min, 0 °C). The supernatant was centrifuged at 50000 × *g* for 15 min. The pellet was suspended in 100 volumes of ice-cold Tris–HCl buffer (50 mM, pH 7.7) and recentrifuged (50000 × *g*, 15 min, 0 °C). The final pellet was resuspended in 150 volumes of ice-cold Tris–HCl buffer (50 mM, pH 7.1) containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 1.1 mM ascorbic acid and 10 μM pargyline, and incubated at 37 °C for 10 min. A portion of this membrane suspension (900 μM) was placed in a tube, and 50 μl of either test compound or vehicle solution was added, followed by 50 μl of [^{3}H]spiperone (40 Ci/mmol) at a final concentration of 0.2 nM. The tubes were incubated at 37 °C for 20 min and filtered through Whatman GF/B glass filters, which were then washed three times with 3 ml of the Tris–HCl buffer (50 mM, pH 7.7). Sulpiride (100 μM) was used for the determination of nonspecific binding. The radioactivity trapped on the filters was measured by liquid scintillation spectrometry. The IC_{50} values were determined from concentration–inhibition curves.

Cisplatin-Induced Emesis The procedure used was a modification of the method of Smith *et al.*[24] Beagle dogs of both sexes weighing 8—14 kg were used. The test drug was injected into a cephalic vein 90 min after the intravenous injection of cisplatin (3 mg/kg). Dogs were then observed for emesis for 5 h after the cisplatin injection.

Doxorubicin and Cyclophosphamide-Induced Emesis The procedure used was a modification of the methods of Bermudez *et al.*[25] Male ferrets weighing 0.8—1.2 kg were used. The test drug was administered intravenously prior to the intravenous injections of doxorubicin (6 mg/kg) followed by cyclophosphamide (80 mg/kg). Ferrets were then observed for emesis for 5 h.

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