Synthesis and Structure−Affinity Relationships of Novel *N*-(1-Ethyl-4-methylhexahydro-1,4-diazepin-6-yl)pyridine-3-carboxamides with Potent Serotonin 5-HT_3 and Dopamine D_2 Receptor Antagonistic Activity

Yoshimi Hirokawa,*,† Iwao Fujiwara,‡ Kenji Suzuki,‡ Hiroshi Harada,† Takashi Yoshikawa,§ Naoyuki Yoshida,§ and Shiro Kato†

Medicinal Chemistry Group and Computational & Structural Chemistry Group, Chemistry Research Laboratories and Discovery Pharmacology II Group, Pharmacology & Microbiology Research Laboratories, Dainippon Pharmaceutical Co. Ltd., Enoki 33−94, Suita, Osaka 564-0053, Japan

Received June 25, 2002

A structurally original series of *N*-(1-ethyl-4-methylhexahydro-1,4-diazepin-6-yl)pyridine-3-carboxamides derived from the corresponding benzamide **5** were prepared and evaluated for their binding affinity for the dopamine D_2 and serotonin 5-HT_3 receptors using rat striatum and rat cortical membrane, respectively. Many of the synthesized pyridine-3-carboxamides exhibited nanomolar binding affinity for the serotonin 5-HT_3 receptor along with moderate to high binding affinity for the dopamine D_2 receptor. Introduction of the more lipophilic bromine atom and methylamino group at the 5- and 6-positions of the pyridine ring, respectively, enhanced the affinity for the dopamine D_2 receptor while keeping a potent serotonin 5-HT_3 receptor binding affinity. As a result of structure−affinity relationships, the 5-bromo-2-methoxy-6-methylaminopyridine-3-carboxamide **53** was selected as the most promising product showing a high binding affinity for both receptors. Compound **53** affinity for the dopamine D_2 and serotonin 5-HT_3 receptors was much more potent than that of metoclopramide (dopamine D_2 receptor; 23.3 nM vs 444 nM, serotonin 5-HT_3 receptor; 0.97 nM vs 228 nM). Optical resolution of the racemate **53** brought about a dramatic change in the pharmacological profile with (*R*)-**53** exhibiting a strong affinity for both the dopamine D_2 and serotonin 5-HT_3 receptors, while the corresponding (*S*)-**53** had a potent serotonin 5-HT_3 receptor binding affinity and a moderate dopamine D_2 receptor binding affinity. X-ray crystallographic study of (*R*)-**53** revealed the existence of two energically stable conformers just like two mirror images. This may account for (*R*)-**53** high affinity for both the dopamine D_2 and serotonin 5-HT_3 receptors. Pharmacologically, (*R*)-**53** [AS-8112] showed a potent antagonistic activity for both the dopamine D_2 and serotonin 5-HT_3 receptors in vivo tests and dose-dependently inhibited both the incidence and frequency of emetic episodes induced by cisplatin (ferrets) and morphine (dogs) with ID_{50} values of 27.1 μg/kg, po and 136 μg/kg, po, respectively. On the basis of this pharmacological profile, (*R*)-**53** is now under further investigation as a potential broad antiemetic agent.

Introduction

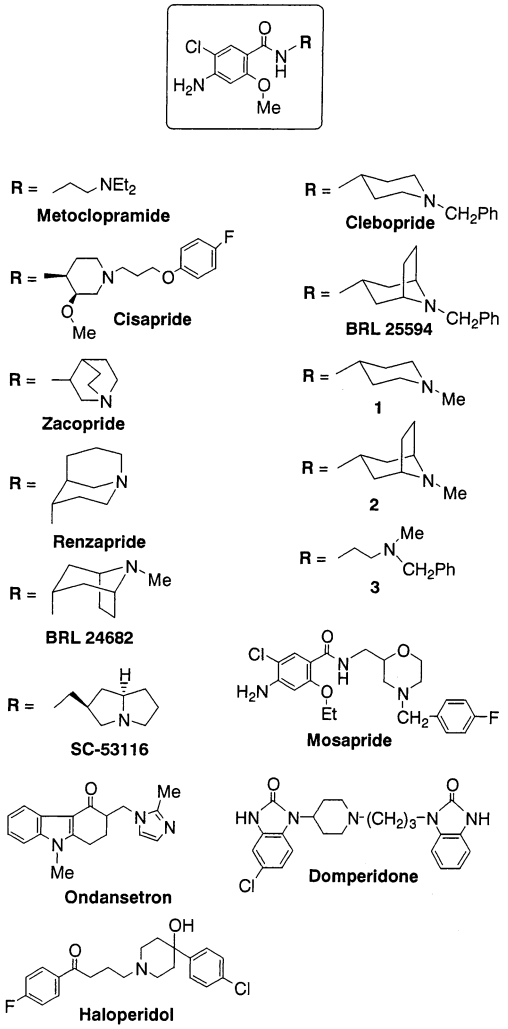
To date, a number of 4-amino-5-chloro-2-methoxybenzamides and other closely related analogues with potent serotoninergic and/or dopaminergic activity have been reported.[1] Although there has been no satisfactory explanation of the unique pharmacological profile of the 4-amino-5-chloro-2-methoxy substitution pattern, this substitution pattern is preferable for activity from circumstantial evidence. The classic and parent benzamide of this family is metoclopramide, which is used clinically as a stimulant of upper gastrointestinal motility and an antiemetic agent.[2,3] Pharmacologically metoclopramide effects are believed to be due to a combination of a relatively weak serotonin (5-hydroxytryptamine) 5-HT_3 (5-HT_3) and dopamine D_2 (D_2) receptors antagonism and a serotonin 5-HT_4 (5-HT_4) receptor agonism.[4] The weak affinity and lack of selectivity of metoclopramide for these receptors can be explained by the large number of permissible conformers arising from a flexible 2-(diethylamino)ethyl moiety. To develop potent 5-HT_3 receptor antagonists and/or 5-HT_4 receptor agonists that are devoid of significant D_2 receptor antagonistic activity, several groups have modified the 2-(diethylamino)ethyl moiety of metoclopramide. Accordingly, benzamides with a conformationally rigid amine framework by cyclization such as piperidine, quinuclidine, and quinolizidine have been prepared.[5] The structures and pharmacological profiles of some of the prepared 4-amino-5-chloro-2-methoxy benzamide derivatives are shown in Chart 1 and Table 1, respectively. Thus, cisapride,[6] mosapride,[7,8] zacopride,[9,10] renzapride,[11] compound **1**,[12] BRL 24682,[13] SC 53116,[14] and compound **2**[12] exhibit good affinity for 5-HT_3 and/or 5-HT_4 receptors, whereas clebopride,[15] BRL 25594,[13] and compound **3**,[13] having a benzyl group on the nitrogen atom in the amine moiety have high affinity for the D_2 receptor.

With the exception of the 5-HT_3 receptor subtype, which is a neuronal receptor coupled directly to a cation

* To whom correspondence should be addressed. Phone: +81-6-337-5902. Fax: +81-6-338-7656. E-mail: yoshimi-hirokawa@dainippon-pharm.co.jp.
† Medicinal Chemistry Group.
‡ Computational & Structural Chemistry Group.
§ Discovery Pharmacology II Group.

10.1021/jm020270n CCC: $25.00
Published on Web 02/01/2003

Chart 1



channel,[16] all serotoninergic and dopaminergic receptor subtypes currently identified belong to the superfamily of G-protein-coupled receptors.[17] Selective 5-HT$_4$ receptor agonists such as mosapride, a gastroprokinetic agent developed in our laboratories (Chart 1), are used to stimulate gastrointestinal motility and are useful in the treatment of a number of gastrointestinal disorders.[18] On the other hand, potent and selective 5-HT$_3$ receptor antagonists such as ondansetron (Chart 1) are clinically effective in the control of nausea and vomiting induced by cancer chemotherapy.[19] Classical neuroleptic agents with a centrally acting D$_2$ receptor antagonistic activity such as the phenothiazines and the butyrophenones are also known to be effective in the control of nausea and vomiting induced by centrally acting emetic stimuli such as antiparkinsonian drugs, loperamide, apomorphine, and morphine.[20] In addition, the traditional antiemetic agent domperidone (Chart 1), a peripheral D$_2$ receptor antagonist, has been shown to be effective for the treatment of chronic upper gastrointestinal distress and the prevention of nausea and vomiting resulting from a variety of causes.[21] However, D$_2$ receptor antagonists are only minimally effective against chemotherapy- or radiation-induced nausea and vomiting.[22,23] Therefore, the combination of a D$_2$ and a 5-HT$_3$ receptors antagonistic activity was seen as a good strategy for the development of effective therapeutic agents for the treatment of nausea and vomiting induced by cancer chemotherapeutic agents, radiation treatment, antiparkinsonian drugs, morphine, and variety of other causes.

Previous work from our laboratories had demonstrated that replacement of the benzyl group in the hexahydro-1,4-diazepine ring of 4-amino-*N*-(1-benzyl-4-methylhexahydro-1,4-diazepin-6-yl)-5-chloro-2-methoxybenzamide (**4**, Chart 2), a potent and selective 5-HT$_3$ receptor antagonist, by an ethyl substituent produces compounds with favorable D$_2$ and 5-HT$_3$ receptors binding affinity profile.[24] The *N*-(1-ethyl-4-methylhexahydro-1,4-diazepin-6-yl)benzamide **5** (Chart 2) exhibited modest D$_2$ receptor binding affinity (IC$_{50}$ = 127 nM) along with a high 5-HT$_3$ receptor binding affinity (IC$_{50}$ = 8.50 nM). Although the structurally novel 1-ethyl-4-methylhexahydro-1,4-diazepine ring of **5** is to some extent conformationally restricted compared with the 2-(diethylamino)ethyl moiety of metoclopramide, it still has some degree of conformational freedom and is thought to be responsible for **5** affinity for both receptors. Therefore, our search for promising compounds possessing high affinity for both the D$_2$ and 5-HT$_3$ receptors began with modification of the benzoyl moiety of **5**.[25,26] Exploration in greater depth of the structural requirements for this dual affinity of **5** resulted in the discovery of the optimal 4-methylamino analogue **6**. After optical resolution of **6**, the (*R*)-enantiomer [(*R*)-**6**] was found to have the highest affinity for the D$_2$ receptor with a potent affinity for the 5-HT$_3$ receptor (Chart 2). In addition, (*R*)-**6** behaved as D$_2$ and 5-HT$_3$ receptors antagonist[27] and did not bind to the 5-HT$_4$ receptor.[28]

It is known that the 3-position in the aromatic moiety of the 4-amino-5-chloro-2-methoxybenzamides is metabolized to produce the corresponding 3-hydroxybenzamides.[29] To avoid this potential metabolism, Coldwell et al. reported the preparation of 6-amino-5-chloro-2-methoxypyridine-3-carboxamides as pyridine analogues of 4-amino-5-chloro-2-methoxybenzamides and showed that the 6-amino-5-chloro-2-methoxypyridine-3-carbonyl moiety is a viable bioisostere of the 4-amino-5-chloro-2-methoxybenzoyl nucleus in the benzamide family of D$_2$ or 5-HT$_3$ receptor antagonists.[30] Accordingly, and as a continuation of our exploratory work on potential broad antiemetic agents with dual D$_2$ and 5-HT$_3$ receptors antagonistic activity, our efforts were focused on further modification of the benzoyl moiety of **5** and (*R*)-**6** and the possibility of the pyridine nucleus being a viable alternative in the D$_2$ and 5-HT$_3$ receptors antagonist series (Chart 2).

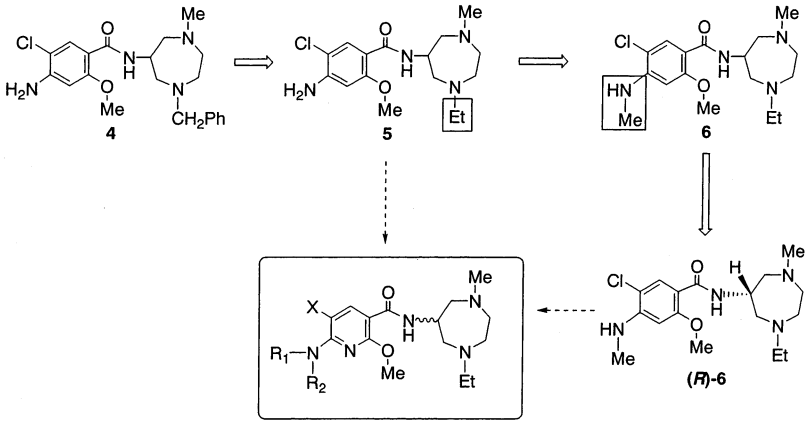
In the present paper, we describe the synthesis of a structurally novel series of *N*-(1-ethyl-4-methylhexahydro-1,4-diazepin-6-yl)pyridine-3-carboxamides and evaluate their structure−affinity relationships (SARs) for the D$_2$ and 5-HT$_3$ receptors. In addition, optical resolution of selected pyridine-3-carboxamides with high affinity for both receptors along with their in vivo pharmacological profiles were examined. Finally, an X-ray crystallographic study of the selected pyridine-3-carboxamide (*R*)-**53** was performed and rationalization of its unique results as regard to the binding affinity for the D$_2$ and 5-HT$_3$ receptors is also discussed.

Table 1. Pharmacological Profile of Several Benzamide Derivatives

	serotonin 5-HT_3 receptor affinity (nM)[a]	serotonin 5-HT_4 receptor affinity (nM)[b]	dopamine D_2 receptor affinity (nM)[c]
metoclopramide	K_i; 210 ± 61	K_i; 546 ± 12	K_i; 303 ± 2.4
cisapride	K_i; 94.7 ± 21.2	K_i; 14.3 ± 1.9	K_i; 227[e]
mosapride	K_i; 1189 ± 191	K_i; 69.9 ± 8.6	K_i; >10000
(*S*)-zacopride	K_i; 0.2 ± 0.04[d]	K_i; 383 ± 64[d]	K_i; >1000[d]
renzapride	K_i; 5.3 ± 59[e]	K_i; 40.4 ± 54[d]	K_i; >10000[e]
1	K_i; 267 ± 59[d]	K_i; 832 ± 54[d]	K_i; >1000[d]
BRL 24682	K_i; 0.8 ± 0.2[d]	K_i; 48 ± 5.6[d]	K_i; >1000[d]
SC-53116	K_i; 152 ± 59[e]	K_i; 21 ± 59[f]	K_i; >10000[e]
2	K_i; 41.8 ± 5.3[d]	K_i; >1000[d]	K_i; >1000[d]
clebopride	K_i; >1000[d,g]	K_i; 104 ± 58[d,g]	K_i; 11.9 ± 3.8[d,g]
BRL 25594	K_i; >1000[d,g]	K_i; 233 ± 60[d,g]	K_i; 0.28 ± 0.04[d,g]
3	K_i; >1000[g]	K_i; 867 ± 71[g]	K_i; 24.3 ± 6.7[g]
4[h]	IC_{50}; 2.07 [33.6−0.127]	IC_{50}; >1000	IC_{50}; >1000
(*R*)-**6**[h]	IC_{50}; 2.86 [603−0.203]	IC_{50}; >1000	IC_{50}; 34.6 [5085−0.348]

[a] Determined in rat cortical membrane using [^{3}H]GR65630. [b] Determined in guinia-pig striatum using [^{3}H]GR113808. [c] Determined in rat striatum using [^{3}H]spiperone. [d] Reference 12. [e] Reference 14. [f] Reference 59. [g] Reference 13. [h] Reference 26.

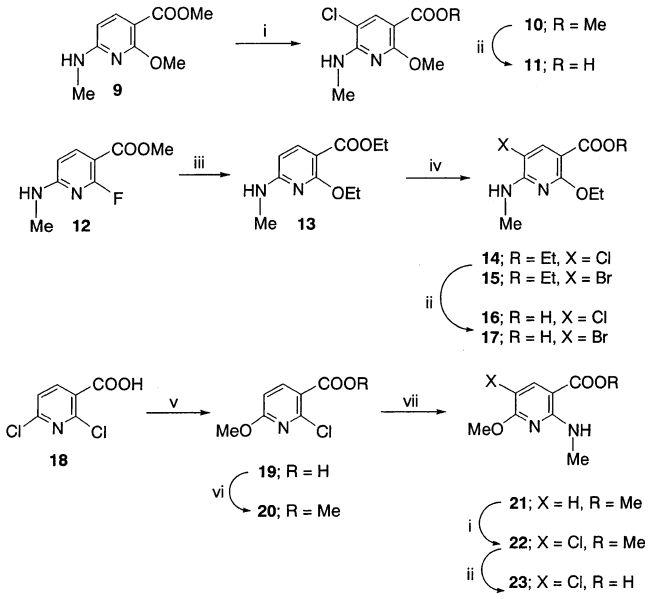
Chart 2



Chemistry

The requisite pyridine-3-carboxylic acid derivatives **11**, **16, 17**, **23**, and **35**−**37** were prepared by the methods shown in Schemes 1 and 2. Reaction of methyl 2-methoxy-6-methylaminopyridine-3-carboxylate[31,32] (**9**) with *N*-chlorosuccinimide (NCS), followed by alkaline hydrolysis of the methyl ester **10** gave 5-chloro-2-methoxy-6-methylaminopyridine-3-carboxylic acid (**11**) in 77% overall yield. The corresponding 2-ethoxypyridine-3-carboxylic acid analogues were prepared as follows. Displacement reaction of the fluorine atom of methyl 2-fluoro-6-methylaminopyridine-3-carboxylate[31] (**12**) with an ethoxide anion produced from potassium *tert*-butoxide and EtOH was accompanied by ester exchange to furnish the 2-ethoxypyridine-3-carboxylic ethyl ester **13** in 80% yield. After chlorination of **13** with NCS or bromination with *N*-bromosuccinimide (NBS), the resulting 5-halogenopyridine-3-carboxylic esters **14** and **15** were hydrolyzed with aqueous NaOH to afford the corresponding carboxylic acids **16** and **17** in good yields. The regioisomer of **11**, 5-chloro-6-methoxy-2-methylaminopyridine-3-carboxylic acid (**23**) was synthesized from the commercially available 2,6-dichloropyridine-3-carboxylic acid (**18**). Treatment of **18** with an excess of methoxide anion produced from potassium *tert*-butoxide and MeOH at 50 °C, followed by methyl esterification via the corresponding acid chloride of the pyridine-3-carboxylic acid **19** gave the 6-methoxypyridine-3-carboxylic ester **20** as main product. On the other hand,

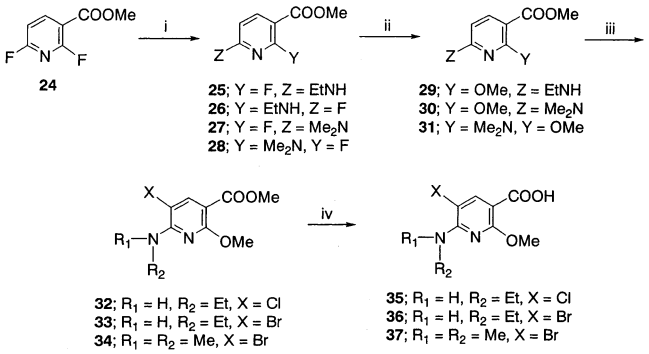
Scheme 1. Preparation of Pyridine-3-carboxylic Acids **11**, **16**, **17**, and **23**[a]



[a] Reagents and conditions: i, NCS, DMF, 80 °C; ii, NaOH, MeOH−H_2O, reflux; iii, ^{t}BuOK, EtOH; reflux, iv, NCS (or NBS), DMF, 80 °C; v, ^{t}BuOK, MeOH, reflux; vi, $SOCl_2$, MeOH, reflux; vii, aq $MeNH_2$, EtOH, reflux.

when **18** underwent a nucleophilic substitution reaction by a methoxide anion in refluxing MeOH for 4 day, the amount of the 2-chloro-6-methoxypyridine-3-carboxylic

Scheme 2. Preparation of 2-Methoxypyridine-3-carboxylic Acids **35**−**37**[a]

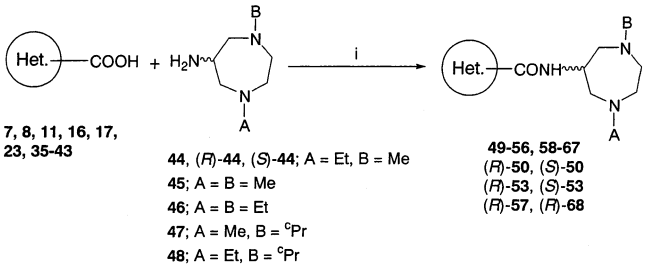


[a] Reagents and conditions: i, $EtNH_2$, EtOH, ca. 5 °C (Me_2NH, EtOH, ca. −25 °C); ii, ^{t}BuOK, MeOH, reflux; iii, NCS and/or NBS, DMF, 80 °C; iv, NaOH, MeOH−H_2O, reflux.

acid **19** increased, and the ester **20** was obtained in 66% overall yield. The resulting ester **20** was treated with methylamine to afford the 6-methoxy-2-methylaminopyridine-3-carboxylic ester **21** in 83% yield. Chlorination of **21** with NCS, followed by alkaline hydrolysis of the ester **22** gave the desired **23** in good yield (Scheme 1). The structure of **20** was confirmed by differential nuclear Overhauser effect (NOE) experiment and comparison with **11** and **23**. Irradiation at δ 4.00 (OMe) of **20** enhanced signal intensity of the adjacent pyridine 5-proton (δ 6.70).

Preparation of the 6-ethylamino- and 6-dimethylaminopyridine-3-carboxylic acids **35**−**37** is shown in Scheme 2. Reaction of the methyl 2,6-difluoropyridine-3-carboxylate[31] (**24**) with 1.0 mol equiv of $EtNH_2$·HCl in DMF in the presence of Et_3N under ice-cooling afforded a mixture of the 6-ethylaminopyridine-3-carboxylic ester **25** and the regioisomer **26**. The mixture was separated by silica gel column chromatography, and the less polar 2-ethylaminopyridine **26** and the more polar 6-ethylaminopyridine **25** were obtained in 31% and 61% yields, respectively. On the other hand, treatment of **24** with ca. 2.2 mol equiv of Me_2NH in EtOH at −20 °C gave a mixture of 6- and 2-dimethylaminopyridines **27** and **28** in 69% yield in a ratio of 5:1. The ratio was determined by ^{1}H NMR spectroscopy. Attempts to separate the mixture of **27** and **28** using silica gel column chromatography were unsuccessful. Recrystallization of the mixture from AcOEt/hexane gave a small amount of the 6-dimethylaminopyridine-3-carboxylic ester **27**. Confirmation of the structure of **25** and **27** was provided by differential NOE experiment. Irradiation at δ 3.3−3.47 (CH_2Me of **25**) and δ 3.15 (NMe_2 of **27**) enhanced signal intensity of the adjacent pyridine 5-protons of **25** and **27**, respectively (δ 6.22 of **25**, δ 6.31 of **27**). Reaction of **25** with a methoxide anion in MeOH gave the 2-methoxypyridine **29** in 82% yield. On the other hand, under similar conditions the mixture of **27** and **28** was treated and worked up to produce a mixture of the 2-methoxypyridine **30** and the regioisomer **31** as a solid. The solid obtained was washed with hexane to afford only the 6-dimethylaminopyridine **30** in 70% yield. Treatment of **29** or **30** with NCS and/or NBS, followed by alkaline hydrolysis of the resulting 5-halogeno-6-ethylaminopyridines **32** and **33**, and 5-bromo-6-dimethylaminopyridine **34** gave the desired pyridine-3-carboxylic acids **35**−**37** in good yields.

Scheme 3. Synthetic Route to Target Carboxamides **49**−**56**, **58**−**67**, (*R*)-**50**, (*S*)-**50**, (*R*)-**53**, (*S*)-**53**, (*R*)-**57**, and (*R*)-**68**[a]



[a] Reagents and conditions: i, EDC, CH_2Cl_2, room temperature.

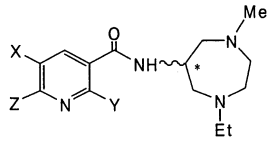
The 1,4-dialkylhexahydro-1,4-diazepinylcarboxamides **49**−**56**, **58**−**67**, (*R*)-**50** and (*S*)-**50**, (*R*)-**53** and (*S*)-**53**, (*R*)-**57**, and (*R*)-**68** were synthesized by reaction of the appropriate carboxylic acids with the 6-amino-1,4-dialkylhexahydro-1,4-diazepines **44**,[26] **45**,[57] **46**,[24] **47**,[26] **48**,[26] (*R*)-**44**,[26,37] and (*S*)-**44**,[26] in the presence of 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDC) as a coupling agent (Scheme 3).

Results and Discussion

Method. The binding affinity values for the 5-HT$_3$ receptor of compounds **49**−**56**, **58**−**67**, (*R*)-**57**, and (*R*)-**68** along with those of each enantiomer of **50** and **53** were determined from each compound ability to displace [^{3}H]GR65630 from its recognition sites in rat cortical membrane according to a previously reported method for [^{3}H]quipazine binding.[24] On the other hand, the affinity for the D$_2$ receptor was evaluated in binding assays by competition for the binding of the radioligand [^{3}H]spiperone, a D$_2$ receptor agonist, to binding sites in rat striatum.[33] The results of these receptor binding assays are listed in Tables 2−4. To characterize binding assays data, the affinity for both receptors of metoclopramide, a potent and selective 5-HT$_3$ receptor antagonist, ondansetron, **5**,[26] and (*R*)-5-chloro-*N*-(1-ethyl-4-methylhexahydro-1,4-diazepin-6-yl)-2-methoxy-4-methylaminobenzamide[26] [(*R*)-**6**], a potent antagonist for the D$_2$ and 5-HT$_3$ receptors, have been included in Table 2.

Structure−Affinity Relationships. As mentioned earlier, it has been reported that the 6-amino-5-chloro-2-methoxypyridine-3-carboxylic acid, a pyridine analogue of the 4-amino-5-chloro-2-methoxybenzoic acid, is a viable bioisostere for benzamides with 5-HT$_3$ or D$_2$ receptor antagonistic activity.[30] Thus, the pyridine analogue of **5**, 6-amino-5-chloro-*N*-(1-ethyl-4-methylhexahydro-1,4-diazepin-6-yl)-2-methoxypyridine-3-carboxamide (**49**) was prepared. The binding affinity of **49** for the 5-HT$_3$ receptor was ca. 1.5-fold higher than that of the parent compound **5**; however, its D$_2$ receptor binding affinity was ca. 2-fold less potent than that of **5**. Moreover, **49** showed a much higher affinity for the 5-HT$_3$ receptor than for the D$_2$ receptor. Replacement of the amino group of **49** by a more lipophilic methylamino group (giving **50**) led to a significant increase in the binding affinity for both receptors, i.e., the affinity for the D$_2$ and 5-HT$_3$ receptors of **50** was ca. 9-fold and 4-fold stronger than that of the 6-amino counterpart **49**, respectively. It is also worth noting that similar results have been reported with the corresponding *N*-(1-ethyl-4-methylhexahydro-1,4-diazepin-6-yl)benzamides.[26] Prepa-

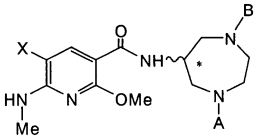
Table 2. Physical Data and Dopamine D_2 and Serotonin 5-HT_3 Receptor Binding Affinity of *N*-(1-Ethyl-4-methylhexahydro-1,4-diazepin-6-yl)pyridine-3-carboxamides



compd	Y	Z	X	*	$[\alpha]^{26}_D$ (MeOH, *c*)	mp, °C (recryst solvent[a])	formula[b]	dopamine D_2 receptor binding affinity, IC_{50} (nM)[c,e]	serotonin 5-HT_3 receptor binding affinity IC_{50} (nM)[d,e]
49	OMe	NH_2	Cl	RS		160−163 (T−H)	$C_{15}H_{24}ClN_5O_2$	386 [6690−22.2]	5.14 [306−0.0861]
50	OMe	NHMe	Cl	RS		157−158 (E)	$C_{16}H_{26}ClN_5O_2 \cdot 2C_4H_4O_4$[f]	43.0 [1900−0.974]	1.26 [32.0−0.0497]
51	OMe	NHEt	Cl	RS		105−108 (M−DE)	$C_{17}H_{28}ClN_5O_2 \cdot 5/2C_2H_2O_4$[g] $\cdot 1/2H_2O$	76.4 [3020−1.94]	1.96 [31.3−0.123]
52	OEt	NHMe	Cl	RS		192−193 (M)	$C_{17}H_{28}ClN_5O_2 \cdot 2C_2H_2O_4$[g]	42.9 [12600−0.146]	0.55 [6.86−0.0434]
53	OMe	NHMe	Br	RS		167−168 (E)	$C_{16}H_{26}BrN_5O_2 \cdot 2C_4H_4O_4$[f]	23.3 [1400−0.388]	0.97 [17.0−0.0555]
54	OMe	NMe_2	Br	RS		172−173 (E)	$C_{17}H_{28}BrN_5O_2 \cdot 5/2C2H_2O_4$[g] $\cdot 1/2H_2O$	75.2 [2240−2.52]	9.93 [1330−0.0739]
55	OMe	NHEt	Br	RS		148−150 (E−IP−PE)	$C_{17}H_{28}BrN_5O \cdot 3/2C_4H_4O_4$[f]	48.1 [8910−0.260]	3.81 [27.5−0.526]
56	OEt	NHMe	Br	RS		131−132 (IP−PE)	$C_{17}H_{28}BrN_5O_2 \cdot 2C_4H_4O_4$[f]	86.4 [9260−0.806]	1.48 [39.9−0.0551]
(*R*)-**57**	NHMe	OMe	Cl	R	+4.4° (1.43)	116−119 (M−DE)	$C_{16}H_{26}ClN_5O \cdot 5/2C_2H_2O_4$[g]	>1000	>100
(*R*)-**50**	OMe	NHMe	Cl	R	−5.1° (1.34)	149−150 (E−DE)	$C_{16}H_{26}ClN_5O_2 \cdot 2C_4H_4O_4$[f]	17.9 [1870−0.171]	1.62 [59.2−0.0411]
(*S*)-**50**	OMe	NHMe	Cl	S	+5.2° (1.09)	148−149 (E−DE)	$C_{16}H_{26}ClN_5O_2 \cdot 2C_4H_4O_4$[f]	202 [9940−4.09]	2.05 [33.6−0.125]
(*R*)-**53**	OMe	NHMe	Br	R	−4.6° (1.10)	152−155 (E)	$C_{16}H_{26}BrN_5O_2 \cdot 2C_4H_4O_4$[f]	6.88 [89.5−0.530]	1.20 [11.9−0.120]
(*S*)-**53**	OMe	NHMe	Br	S	+4.6° (1.10)	154−155 (E)	$C_{16}H_{26}BrN_5O_2 \cdot 2C_4H_4O_4$[f]	122 [6620−2.25]	1.28 [18.4−0.0833]
5								127 [3092−10.5]	8.50 [84.2−0.413]
(*R*)-**6**								34.6 [5085−0.348]	2.86 [60.3−0.203]
ondansetron								>1000	1.54 [46.1−0.0516]
metoclopramide								444 [5430−36.4]	228 [2790−18.7]

[a] Abbreviation for the solvents used are as follows: T = toluene, H = hexane, E = ethanol, M = methanol, DE = diethyl ether, IP = 2-propanol, PE = petroleum ether. [b] All compounds were analyzed for C, H, N, and halogen; analytical results were within ±0.4% for the theoretical values. [c] Determined in rat brain synaptic membrane using [^{3}H]spiperone. [d] Determined in rat brain cortical membrane using [^{3}H]GR65630. [e] Each value represents the mean (95% C.L.). IC_{50} values (the concentration causing 50% inhibition of specific radioligand binding) were expressed in nM and were determined by linear regression analysis (Probit analysis). [f] Fumaric acid. [g] Oxalic acid.

Table 3. Physical Data and Dopamine D_2 and Serotonin 5-HT_3 Receptor Binding Affinity of 2-Methoxy-6-methylamino-*N*-(methylhexahydro-1,4-diazepin-6-yl)pyridine-3-carboxamides



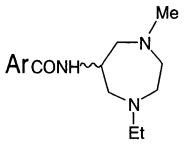
compd	X	A	B	*	mp, °C (recryst solvent[a])	formula[b]	dopamine D_2 receptor binding affinity IC_{50} (nM)[c,e]	serotonin 5-HT_3 receptor binding affinity IC_{50} (nM)[d,e]
58	Cl	Me	Me		128−130 (E-PE)	$C_{15}H_{24}ClN_5O_2$	137 [3740−4.98]	1.30 [35.6−0.0474]
59	Cl	Et	Et		188−189 (M)	$C_{17}H_{28}ClN_5O_2 \cdot 2C_2H_2O_4$[f]$\cdot 1/2H_2O$	27.8 [1540−0.503]	0.65 [26.9−0.0156]
60	Br	Et	Et		199−200 (M)	$C_{17}H_{28}BrN_5O_2 \cdot 5/2C_2H_2O_4$[f]	20.9 [1390−0.313]	0.59 [12.9−0.0268]
61	Br	Me	^{c}Pr	*RS*	158−159 (M)	$C_{17}H_{26}BrN_5O_2 \cdot 5/2C_2H_2O_4$[f]$\cdot 3/4H_2O$	47.1 [1650−1.34]	0.76 [28.0−0.0205]
62	Br	Et	^{c}Pr	*RS*	165−166 (M)	$C_{18}H_{28}BrN_5O_2 \cdot 5/2C_2H_2O_4$[f]	12.9 [1200−0.137]	0.69 [23.1−0.0205]

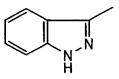
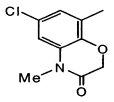
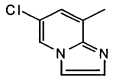
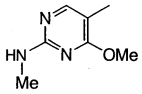
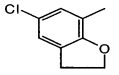
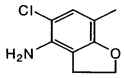
[a] Abbreviation for the solvents used are as follows: E = ethanol, DE = diethyl ether, M = methanol, EA = ethyl acetate. [b−e] See footnotes *b*−*e* in Table 2. [f] Oxalic acid.

ration of the 6-ethylamino analogue **51**, on the other hand, did not provide any improvement in affinity for both receptors compared to the 6-methylamino analogue **50**. Replacement of the 2-methoxy group of **50** by an ethoxy group (giving **52**) caused a ca. 2-fold increase in affinity for the 5-HT_3 receptor, but the affinity for the D_2 receptor did not increase (42.9 nM vs 43.0 nM). The IC_{50} value (0.55 nM) for the 5-HT_3 receptor of **52** was ca. 3 times more than that (1.54 nM) of ondansetron and much stronger than that (228 nM) of metoclopramide.

The influence of a substitution at the 5-position in the pyridine ring of **50** was next studied. Replacement of the chlorine atom of **50** by a bromine atom (giving **53**) caused a 2-fold increase in affinity for the D_2 receptor along with a potent 5-HT_3 receptor binding affinity. The 5-bromopyridine-3-carboxamides **54**−**56** having 6-dimethylamino, 6-ethylamino, and 2-ethoxy groups, respectively, did not display high binding affinity for the D_2 receptor compared to **53**, but maintained a strong 5-HT_3 receptor binding affinity. However, the regioisomer of the pyridine ring of **50**, the (*R*)-5-chloro-6-methoxy-2-methylaminopyridine-3-carboxamide [(*R*)-**57**], showed no affinity for either receptor under investigation. It has been reported that a hydrogen bond between the amidic NH and 2-alkoxy groups in the 4-amino-5-chloro-2-methoxybenzamides holds the amide and the aromatic ring 'in plane', forming a 'virtual ring'.[34,35] In addition, it has been suggested that this hydrogen bond is essential for the D_2 receptor antagonistic activity and may be required for interaction with the 5-HT_3 receptor.[36] It is, therefore, assumed that the hydrogen bond not only stabilizes the orientation of the amide group, but also affects the electronic distribution in the aromatic ring and amide linkage. These results indicate that the best substituent at the 2-, the 6-, and

Table 4. Physical Data and Dopamine D$_2$ and Serotonin 5-HT$_3$ Receptor Binding Affinity of *N*-(1-Ethyl-4-methylhexahydro-1,4-diazepin-6-yl)carboxamides



compd	Ar	mp, °C (recryst solvent[a])	formula[b]	dopamine D$_2$ receptor binding affinity IC$_{50}$ (nM)[c,e]	serotonin 5-HT$_3$ receptor binding affinity IC$_{50}$ (nM)[d,e]
63		198-200 (IP-E)	$C_{16}H_{23}N_5O\cdot 2C_2H_2O_4$[f]	>1000	6.56 [185-0.233]
64		110-113 (C-DE)	$C_{18}H_{25}ClN_4O_3$	>1000	38.7 [1221-1.89]
65		120-122 (E-DE)	$C_{16}H_{22}ClN_5O\cdot 5/2C_2H_2O_4$[f] $\cdot 1/4$EtOH[g]	>1000	148 [816-26.7]
66		171-173 (E-DE)	$C_{15}H_{26}N_6O_2\cdot 5/2C_2H_2O_4$[f] $\cdot 1/4H_2O$	>1000	157 [1620-15.2]
67		89-91 (C-DE)	$C_{17}H_{24}ClN_3O_2$	>1000	29.9 [1560-0.573]
(*R*)-68[h]		167-168 (M-W)	$C_{17}H_{25}ClN_4O_2$	637 [7962-56.7]	3.70 [44.3-0.429]

[a] Abbreviation for the solvents used are as follows: IP = 2-propanol, E = ethanol, C = chloroform, DE = diethyl ether, M = methanol, W = H_2O. [b−e] See footnotes *b*−*e* in Table 2. [f] Oxalic acid. [g] The presence of ethanol was confirmed by ^{1}H NMR spectra. [h] (*R*)-6-Amino-1-ethyl-4-methylhexahydro-1,4-diazepine was used as an amine.

the 5-positions in the pyridine ring is a methoxy, a methylamino, and a bromine, respectively, as in the case of a series of *N*-(1-ethyl-4-methylhexahydro-1,4-diazepin-6-yl)benzamides.[26]

Next, optical resolution of the enantiomers of 5-chloro-*N*-(1-ethyl-4-methylhexahydro-1,4-diazepin-6-yl)-2-methoxy-6-methylaminopyridine-3-carboxamide (**50**) and its 5-bromo counterpart **53**, both of which have high affinity for the D$_2$ and 5-HT$_3$ receptors, was carried out. These enantiomers [(−)-**50** and (−)-**53** and (+)-**50** and (+)-**53**] are known to have absolute (*R*)- and (*S*)-configurations at the C$_6$-carbon atom of the hexahydro-1,4-diazepine ring, respectively, on the basis of asymmetric synthesis of the corresponding amines.[26,37] The binding affinity for the D$_2$ receptor of the (*R*)-enantiomers of **50** and **53** [(*R*)-**50** and (*R*)-**53**] was ca. 2.5−3.5-fold higher than that of the corresponding racemates. The IC$_{50}$ value (17.9 nM) for the D$_2$ receptor of (*R*)-**50** was ca. 2 times more than that (34.6 nM) of the corresponding benzamide (*R*)-**6**. The binding affinity of the 5-bromo analogous (*R*)-**53** of (*R*)-**50** for the D$_2$ receptor was ca. 2.5-fold higher than that of (*R*)-**50**. (*R*)-**53** conferred the highest binding affinity for the D$_2$ receptor in this series. On the other hand, the affinity for the 5-HT$_3$ receptor of these (*R*)-enantiomers was approximately the same as that of the corresponding racemates. IC$_{50}$ value (1.62 nM) for the 5-HT$_3$ receptor of (*R*)-**50** was 1.8-fold stronger than that (2.86 nM) of the benzamide (*R*)-**6**. Although the (*S*)-enantiomers [(*S*)-**50** and (*S*)-**53**] exhibited weak or moderate affinity for the D$_2$ receptor, they retained a strong 5-HT$_3$ receptor binding affinity. The both binding affinities of (*S*)-**53** were ca. 1.5-fold higher than those of (*S*)-**50**. The IC$_{50}$ value (1.28 nM) for the 5-HT$_3$ receptors of (*S*)-**53** was the same as that of ondansetron or (*R*)-**53**. The results as shown in Table 2 are similar to those of a series of *N*-(1-ethyl-4-methylhexahydro-1,4-dizepin-6-yl)benzamides.[26] These findings indicate that the *R*-configuration as amine moiety including the methylamino group, the methoxy group, and the bromine atom in the 3-pyridinecarbonyl moiety are essential for high binding affinity for both the D$_2$ and 5-HT$_3$ receptors and may share the similar binding sites in these receptors.

The influence of a substitution at the 1- and 4-positions in the hexahydro-1,4-diazepine ring of **50** and **53** on the D$_2$ and 5-HT$_3$ receptor binding affinity was next studied (**58**−**62** in Table 3). The 1,4-dimethylhexahydro-1,4-diazepine counterpart **58** of **50** showed a 3-fold decreased binding affinity for the D$_2$ receptor, but maintained a strong 5-HT$_3$ receptor binding affinity. On the other hand, the 1,4-diethylhexahydro-1,4-diazepinylcarboxamide **59** and the 5-bromo analogue **60** compared to **50** and **53**, respectively, displayed an increased affinity for both the D$_2$ and 5-HT$_3$ receptors. Replacement of the ethyl group in **53** by a cyclopropyl group (yielding **61**) resulted in no remarkable change in the binding affinity for the 5-HT$_3$ receptor. The affinity for the D$_2$ receptor was ca.. 2-fold weaker than that of **53**.

Table 5. Inhibition of Apomorphine-Induced Emesis of **51**, **52**, **54**, **58**−**62**, (*R*)-**50**, (*R*)-**53**, and (*S*)-**53**

compd	inhibition of apomorphine-induced emesis[a] 1.0 mg/kg, po (%) [ED_{50}: mg/kg, po]
51	42
52	40
54	83
(*R*)-**50**	52
(*S*)-**53**	47
(*R*)-**53**	100 [0.12]
58	20
59	53
60	69
61	14
62	25
(*R*)-**6**	100 [0.13]
metoclopramide	100 [0.45]

[a] See Experimental Section.

However, the 1-cyclopropyl-4-ethylhexahydro-1,4-diazepine congener **62** exhibited strong binding affinity for both receptors; affinity for the D_2 and 5-HT_3 receptors was ca. 2-fold and 1.5-fold higher than those of the parent **53**, respectively. These results suggest that there is a limitation to the substituent size in the hexahydro-1,4-diazepine ring which can fit into the D_2 receptor binding site and indicate that the ethyl group may be essential for favorable binding affinity for the D_2 receptor. Unlike the D_2 receptor, the 5-HT_3 receptor binding site for substituents in the hexahydro-1,4-diazepine ring is though to have a more tolerant pocket with a volume for a methyl, ethyl, cyclopropyl, butyl,[26] or benzyl[26] substituent.

Finally, the SARs associated with modification of the pyridinylcarboxamide moiety, while keeping the 1-ethyl-4-methylhexahydro-1,4-diazepine ring constant were examined. As shown in Table 4, replacement of the 6-amino-5-chloro-2-methoxypyridine ring of **49** by 1*H*-indazole, 6-chloro-3,4-dihydro-4-methyl-3-oxo-2*H*-1,4-benzoxazine, 6-chloroimidazo[1,2-*a*]pyridine ring, and 5-chloro-2,3-dihydrobenzo[*b*]benzofuran rings, which are aromatic moieties of highly potent and selective 5-HT_3 receptor antagonists, or by 4-methoxy-2-methylaminopyrimidine ring, which is an aromatic moiety of a potent D_2 receptor antagonist, or 4-amino-5-chloro-2,3-dihydrobenzo[*b*]benzofuran ring, which is an aromatic moiety of a potent and selective 5-HT_4 receptor agonist caused a remarkable decrease in affinity for the D_2 receptor. Only compounds **63** and (*R*)-**68** exhibited a high affinity for the 5-HT_3 receptor. From these results, it was assumed that instead of using the 4-amino-5-chloro-2-methoxybenzamide as nucleus, 1*H*-indazole and 4-amino-5-chloro-2,3-dihydrobenzo[*b*]benzofuran rings could be used as nucleus of compounds with strong 5-HT_3 receptor affinity. However, it was later confirmed that the pharmacophores for a high D_2 receptor affinity are the benzene and pyridine rings with the 5-chloro-(bromo)-2-methoxy-4(6)-methylamine substitution pattern as an aromatic moiety.

Pharmacological Activity. On the basis of SARs, the pyridine-3-carboxamides **51**, **52**, **54**, (*R*)-**50**, (*R*)-**53**, (*S*)-**53**, and **58**−**62** with a moderate to potent D_2 receptor binding affinity along with a high 5-HT_3 receptor binding affinity were selected for further in vivo biological assays involving inhibition of apomorphine (1.0 mg/kg, po)-induced emesis in dogs.[38] As shown in Table 5, only (*R*)-**53** completely inhibited apomorphine-induced emesis with an ED_{50} value of 0.12 mg/kg, po. This ED_{50} value was ca. 4-fold stronger than that of metoclopramide (0.45 mg/kg, po) and equal to that of (*R*)-**6** (0.13 mg/kg, po). Although other compounds [**51**, **52**, **54**, (*R*)-**50**, (*S*)-**53**, and **58**−**62**] affinity for the D_2 receptor was higher than that of metoclopramide, they did not completely inhibit apomorphine-induced emesis. These results indicate that (*R*)-**53** has a potent D_2 receptor antagonistic activity in dogs and is orally bioavailable like (*R*)-**6** and metoclopramide. It is well-known that 5-HT_3 receptor antagonists block the bradycardia (von Bezold−Jarisch reflex) induced by 2-methylserotonin, a receptor agonist that mediates the activation of the 5-HT_3 receptor located on vagal afferent fibers in cardiac ventricles and is widely used to assay 5-HT_3 blocking activity in vivo.[39−41] Compound (*R*)-**53** like (*R*)-**6**, ondanstron, and metoclopramide dose-relately inhibited the 2-methylserotonin-induced bradycardia in rats with IC_{50} values of 2.3, 1.4, 2.8, and 860 μg/kg, iv, respectively. In addition, the potency of these compounds was in accordance with that of their affinity for the 5-HT_3 receptor in the rat frontal cortex. These findings suggest that (*R*)-**53** may be classified as a potent 5-HT_3 receptor antagonist both in vitro and in vivo. On the whole, the optically active (*R*)-*N*-(1-ethyl-4-methylhexahydro-1,4-diazepin-6-yl)benzamide [(*R*)-**6**] and the corresponding pyridine-3-carboxamide [(*R*)-**53**] were found to possess the most favorable activity profile. As for compounds (*R*)-**6**, (*R*)-**53**, (*S*)-**53**, ondanstron, metoclopramide, and domperidone binding affinity for other dopamine and serotonin receptor subtypes, the results are shown in Table 6. All compounds showed much weak affinity for the 5-HT_4 receptor while the affinity for the dopamine D_3 (D_3) receptor varied considerably with (*R*)-**53** and domperidone exhibiting the most potent affinities (IC_{50} = 1.13 nM and 2.68 nM, respectively).

Regarding the role of central dopaminergic mechanisms in emesis, it is well-known that D_2 receptors in the area postrema play an important role in the regulation of emetic responses in humans, ferrets, and dogs.[42,43] Moreover, Yoshikawa et al. have recently reported that (*R*)-7-hydroxy-2-(*N*,*N*-di-*n*-propylamino)tetralin [(*R*)-7-OH-DPAT], a selective D_3 receptor agonist, elicits emesis in ferrets and dogs.[44] Experiments with ferrets have also revealed that (*R*)-7-OH-DPAT-induced emesis may be mediated by the D_3 receptor located in the area postrema, which is the locus of the chemoreceptor trigger zone.[44] It can, therefore, be assumed that both the D_2 and D_3 receptors in the area postrema play an important role in the regulation of emesis in ferrets. As mentioned above, (*R*)-**6**, (*S*)-**53**, and metoclopramide showed high to moderate binding affinity for the D_3 receptor with decreasing order, i.e., (*R*)-**53** > domperidone ≫ (*S*)-**53** > (*R*)-**6** ≫ metoclopramide. On the other hand, although all compounds except domperidone and metoclopramide displayed a high binding affinity for the 5-HT_3 receptor at a range of IC_{50} = 1.20−2.86 nM. (*R*)-**53** was also found to bind to mixed D_2 and D_3[45] and 5-HT_3 receptors and showed potent antagonistic activity for all these receptors. From these results, (*R*)-**53** [AS-8112] was selected as the most optimum compound for further investigation. Next, (*R*)-**53** was tested for dose−response suppression of cisplatin-induced emesis in

Table 6. Dopamine D_2 and D_3 and Serotonin 5-HT_3 Receptor Binding Affinity of (*R*)-**6**, (*R*)-**53**, (*S*)-**53**, Metoclopramide, Ondansetron, and Domperidone

	receptor binding affinity IC_{50} (nM)			
compd	dopamine D_2[a]	dopamine D_3[b]	serotonin 5-HT_3[c]	serotonin 5-HT_4[d]
(*R*)-**6**	34.6 [5058−0.348]	15.1 [153−1.49]	2.86 [60.3−0.203]	>10000
(*R*)-**53**	6.88 [89.5−0.530]	1.13 [10.1−0.127]	1.20 [11.9−0.120]	>10000
(*S*)-**53**	122 [6620−2.25]	14.3 [95.4−2.16]	1.28 [18.4−0.0833]	>1000
metoclopramide	444 [5430−36.4]	61.3 [677−5.54]	228 [2790−18.7]	912 [5850−142]
ondansetron	>10000	>10000	1.54 [46.1−0.0516]	>10000
domperidone	13.6 [23.7−0.786]	2.68 [120−0.0598]	>1000	>1000

[a] See footnotes c,e in Table 2. [b] Determined in rat striatum using [^{3}H](*R*)-7-OH-DPAT. [c] See footnotes d,e in Table 2. [d] Determined in guinea-pig striatum using [^{3}H]GR113808.

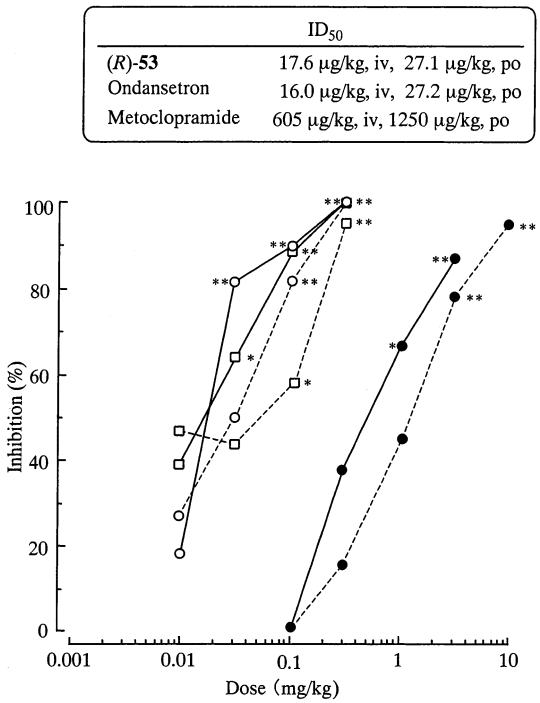


Figure 1. Effects of (*R*)-**53**, metoclopramide, and ondansetron on cisplatin-induced emesis in ferrets. ○, (*R*)-**53**; ●, metoclopramide; □, ondansetron. Solid line, intravenous administration; dotted line, oral administration. Test compounds were administrated immediately (iv) or 30 min (po) before treatment of cisplatin (10 mg/kg, iv). Emetic episodes were observed for 4 h after treatment of cisplatin. *: $p < 0.05$, **: $p < 0.01$ compared with the control group ($N = 12$).

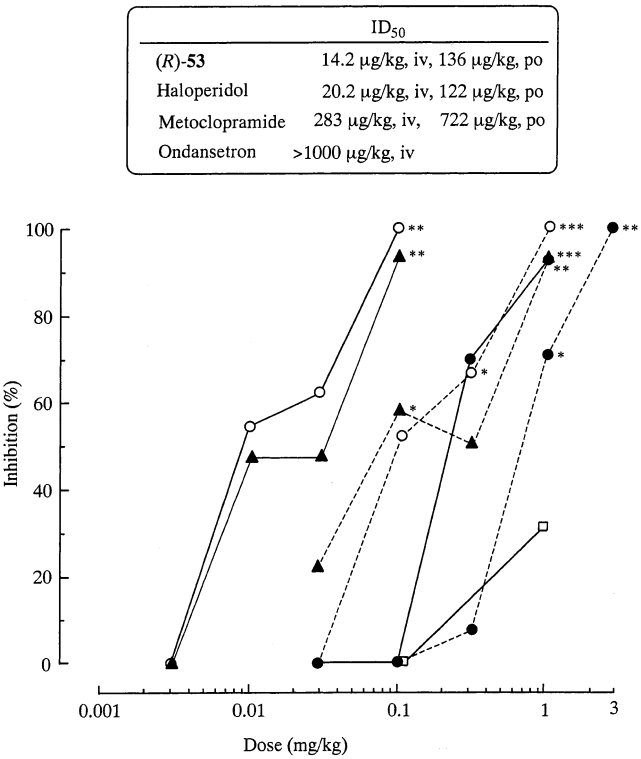


Figure 2. Effects of (*R*)-**53**, haloperidol, metoclopramide, and ondansetron on morphine-induced emesis in dogs. ○, (*R*)-**53**; ▲, haloperidol; ●, metoclopramide; □, ondansetron. Solid line, intravenous administration; dotted line, oral administration. Test compounds were administrated 15 min (iv) or 60 min (po) before treatment of morphine (3 mg/kg, sc). Emetic episodes were observed for 30 min after treatment of morphine. *: $p < 0.05$, **: $p < 0.01$, ***$p < 0.001$ compared with the control group ($N = 11$ or 12).

ferrets and morphine-induced emesis in dogs (Figures 1 and 2). As for the cisplatin-induced emesis, it is known that emesis is mainly mediated by stimulation of abdominal visceral afferent nerves via the 5-HT_3 receptor.[43,46] In addition, it has been shown that the central 5-HT_3 receptor, which is mainly located in the area postrema, is also important in emetic responses.[40,47,48] However, cisplatin-induced emesis has been shown not to be mediated via the D_2 or D_3 receptor.[49] In the present study, (*R*)-**53,** ondansetron, and metoclopramide dose-dependently inhibited emesis in ferrets with ID_{50} values of 17.6 μg/kg, iv, 16.0 μg/kg, iv, and 605 μg/kg, iv, respectively. Moreover, oral administration of these compounds significantly inhibited emesis in ferrets with ID_{50} values of 27.1 μg/kg, po, 27.2 μg/kg, po, and 1250 μg/kg, po, respectively (Figure 1). The activity of (*R*)-**53** was comparable to that of ondansetron, a selective and potent 5-HT_3 receptor antagonist, and was much higher than that of metoclopramide.

Morphine is a well-known emetogenic agent in human. Presently, dopamine receptor antagonists such as phenothiazines, butyrophenones, and benzamides, which have affinity for the D_2 and D_3 receptors, are used as antiemetic agents. In this study, both (*R*)-**53** and a D_2 receptor antagonist haloperidol (Chart 1), a butyrophenone derivative, significantly inhibited morphine-induced emesis in dogs with ID_{50} values of 14.2 μg/kg, iv, and 20.2 μg/kg, iv, respectively (Figure 2). The antiemetic effect of (*R*)-**53** was as potent as that of haloperidol and ca. 20-fold stronger than that of metoclopramide (283 μg/kg, iv). On the other hand, ondansetron, a 5-HT_3 receptor antagonist, did not cause 50% inhibition even at the high dose of 1 mg/kg, iv. In addition, (*R*)-**53**, haloperidol, and metoclopramide, administered orally, inhibited the morphine-induced eme-

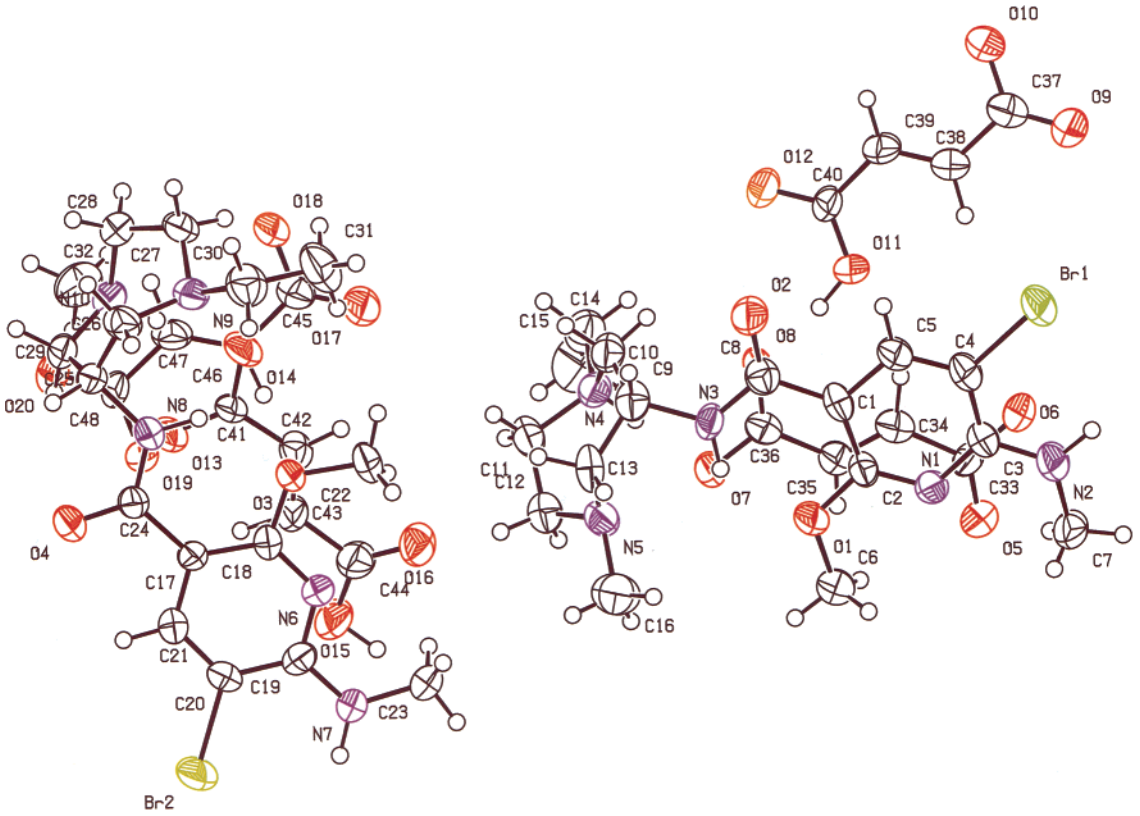


Figure 3. ORTEP diagram with 50.0% probability ellipsoids obtained from X-ray structure of (*R*)-**53** showing atom numbering and two conformations in crystal.

sis in dogs with ID_{50} values of 136 μg/kg, po, 122 μg/kg, po, and 722 μg/kg, po, respectively (Figure 2).

In the present study, metoclopramide, which has weak affinity for the D_2, D_3, and 5-HT_3 receptors, inhibited the emesis triggered by cisplatin and morphine, however, the potency of this inhibition was weak. On the other hand, (*R*)-**53** blocked or significantly reduced vomiting and retching in ferrets given cancer chemotherapeutic agents such as cisplatin, cyclophosphamide,[45] and doxorubicin.[45] Additionally, (*R*)-**53** also blocked emetic episodes induced by morphine and apomorphine[45] in dogs. From the results above, (*R*)-**53** is a potent D_2, D_3, and 5-HT_3 receptors antagonist with potent activity both in vitro and in vivo and is, therefore, expected to be a broad antiemetic agent.

X-ray Structure Determination of (*R*)-53. To clarify simultaneously strong binding affinity for two different D_2 and 5-HT_3 receptors of one enantiomer (*R*)-**53**, X-ray crystallography was carried out. First, the crystal structure of (*R*)-**53** and its molecular conformations were determined (Figure 3). Interestingly, two molecules with different conformations in an asymmetric unit cell of a single crystal were observed (Figure 3). For convenience in the following discussion, the two conformational isomers are designated as conformer **A** (right molecule in Figure 3) and **B** (left molecule in Figure 3). To confirm the stability of the two conformational isomers, molecular orbital calculations were performed and the molecular energies were compared. Structural comparison between the two conformations were carried out using computer graphics. Energy optimized structures of each conformer were almost the same as the solid state structure and the difference in energy values between conformer A and B was only 0.25 kcal/mol by 3-21G* basis set.[50] The geometrical relationship between conformers **A** and **B** seemed just like each enantiomer, i.e., conformers **A** and **B** were almost mirror images of each other except for the positions of the methyl and ethyl groups on the hexahydro-1,4-diazepine rings as shown in Figure 4. Conformer **A** or **B** could convert into conformer **B** or **A** by rotation around the bonds C_1-C_8, N_3-C_9, and $C_{11}-C_{12}$ (see dihedral angles in Supporting Information). It is assumed that that the conformers are interchangeable in solution and that the methyl and ethyl groups in the hexahydro-1,4-diazepine ring are interchanged and flipped in pseudo mirror plane in space as shown in Figure 4. According to this interconversion, the roles of nitrogen atoms on the hexahydro-1,4-diazepine ring in the pharmacophore for 5-HT_3 and D_2 receptors may be interchangeable.

On the basis of this hypothesis and the results of SARs, the methyl group in the hexahydro-1,4-diazepine ring of one conformation may play an important role on the 5-HT_3 receptor binding profile of (*R*)-**53**. On the other hand, the ethyl group of the other conformation may participate in the potent affinity for the D_2 receptor.

Conclusion

The benzene ring of the *N*-(1-ethyl-4-methylhexahydro-1,4-diazepin-6-yl)benzamide of **5** and **6**, two potent D_2 and 5-HT_3 receptors antagonists, can be replaced by an aromatic isostere, pyridine ring without seriously affecting the affinity for both receptors, i.e., the 6-amino-5-chloro-2-methoxy substitution pattern of the pyridine ring like the corresponding benzene ring were found to be essential for affinity toward the dopamine and serotonin receptors. The ethyl substituent of hexahydro-

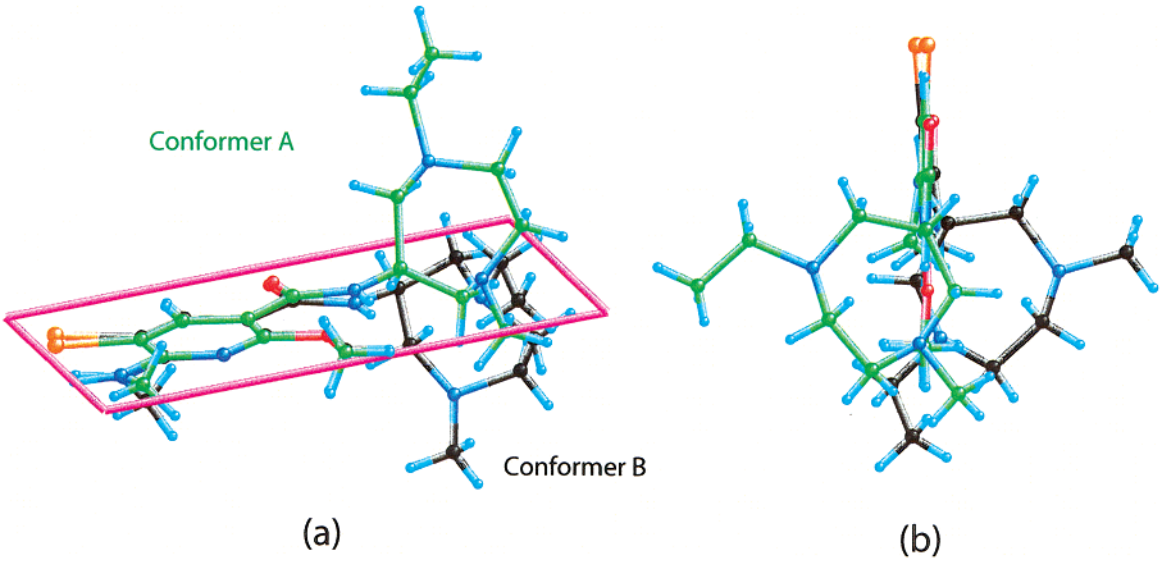


Figure 4. Orthogonal perspective view of overlaid two conformations of (*R*)-**53** in crystal: (a) The box by bold magenta lines denotes the plane of the pyridine ring. (b) A view of symmetrical hexahydro-1,4-diazepine rings of two conformations. The six atoms in the pyridine ring were subjected to a least-squares fit.

1,4-diazepine ring plays an important role in the binding of compounds to the D$_2$ receptor, since replacement of this substituent by other groups affects the affinity for this receptor. On the other hand, the 5-HT$_3$ receptor tolerates a more structural diversity of the hexahydro-1,4-diazepine ring. The enantiomers of *N*-(1-ethyl-4-methylhexahydro-1,4-diazepin-6-yl)benzamides, and the corresponding pyridine-3-carboxamides were shown to possess different pharmacological profile: (*R*)-enantiomers appeared to block both the D$_2$ and 5-HT$_3$ receptors, while (*S*)-enantiomers displayed a potent antagonistic activity for 5-HT$_3$ receptor only. The essential factors for a strong and simultaneous affinity toward the D$_2$, D$_3$, and 5-HT$_3$ receptors can be summarized as follows: (a) 4(6)-amino-5-chloro-2-methoxy substitution pattern of aromatic moiety, (b) hexahydro-1,4-diazepine ring as an amine moiety, (c) the ethyl group at the 1-position and the methyl, ethyl, and cyclopropyl groups at the 4-position in hexahydro-1,4-diazepine ring, and (d) *R* configuration at the 6-position of hexahydro-1,4-diazepine ring. Starting from the high affinity and selectivity for the 5-HT$_3$ receptor shown by the *N*-(1-benzyl-4-methylhexahydro-1,4-diazepin-6-yl)benzamide **4**, our work has resulted in the discovery of (*R*)-**53**, a novel D$_2$, D$_3$, and 5-HT$_3$ receptors antagonist. (*R*)-**53** is, therefore, expected to be a broad antiemetic agent.

Experimental Section

All melting points were determined on a Yanagimoto micromelting point apparatus without correction. IR spectra were recorded on a Shimadzu FTIR-8200PC spectrometer with KBr disks unless otherwise stated. Electron ionization and atmospheric pressure chemical ionization mass spectra were obtained on a JEOL JMS D-300 and Hitachi M-1000 spectrometer, respectively. ^{1}H NMR spectra were recorded on a Varian Gemini-200 (200 MHz) or a JEOL JNM-LA300 (300 MHz) spectrometer using dilute solution in $CDCl_3$ unless otherwise stated. Chemical shifts were expressed as δ (ppm) value from tetramethylsilane as an internal standard and coupling constants (*J*) are given in Hz. Optical rotations were measured at 589 nm with a Jasco P-1020 digital polarimeter. Analytical HPLC was performed with a Shimadzu LC-6A, SPD-6A instruments. Organic extracts were dried over anhydrous $MgSO_4$. The solvents were evaporated under reduced pressure. Flash chromatography was carried out on 60 μm mesh silica gel (Fuji Silysia FL60D). The following known carboxylic acids and 6-amino-1,4-dialkylhexahydro-1,4-diazepines were prepared according to the cited literature: 6-amino-5-chloro-2-methoxypyridine-3-carboxylic acid[30] (**7**), 5-bromo-2-methoxy-6-methylaminopyridine-3-carboxylic acid[31,32] (**8**), 1*H*-indazole-3-carboxylic acid[51] (**38**), 6-chloro-3,4-dihydro-4-methyl-3-oxo-2*H*-1,4-benzoxazine-8-carboxylic acid[52] (**39**), 6-chloroimidazo[1,2-*a*]pyridine-8-carboxylic acid[53] (**40**), 4-methoxy-2-methylaminopyrimidine-5-carboxylic acid[54] (**41**), 5-chloro-2,3-dihydrobenzo[*b*]furan-7-carboxylic acid[55] (**42**), 4-amino-5-chloro-2,3-dihydrobenzo[*b*]furan-7-carboxylic acid[56] (**43**), (*RS*)-, (*R*)-, and (*S*)-6-amino-1-ethyl-4-methylhexahydro-1,4-diazepines[26,37] [**44**, (*R*)-**44**, (*S*)-**44**], 6-amino-1,4-dimethylhexahydro-1,4-diazepine[57] (**45**), 6-amino-1,4-diethylhexahydro-1,4-diazepine[24] (**46**), 6-amino-1-cyclopropyl-4-methylhexahydro-1,4-diazepine[26] (**47**), and 6-amino-1-cyclopropyl-4-ethylhexahydro-1,4-diazepine[26] (**48**).

Methyl 5-Chloro-2-methoxy-6-methylaminopyridine-3-carboxylate (10). A solution of 2-methoxy-6-methylaminopyridine-3-carboxylic acid (**9**, 1.1 g, 5.6 mmol) and *N*-chlorosuccinimide (NCS, 0.82 g, 6.1 mmol) in DMF (10 mL) was heated at 80 °C for 4 h. The reaction mixture was poured into ice−water, and the resulting precipitate was collected by filtration, washed with water, and dried to give 1.1 g (85%) of **10**, mp 118−119 °C (AcOEt/hexane). ^{1}H NMR δ: 3.09 (d, 3H, J = 4.7), 3.82 (s, 3H), 4.02 (s, 3H), 5.34 (br., 1H), 8.01 (s, 1H). MS *m*/*z*; 231 (MH^+). IR cm^{-1}; 3404, 3366, 2949, 1713, 1600, 1570, 1265, 1232. Anal. ($C_9H_{11}ClN_2O_3$) C, H, N, Cl.

5-Chloro-2-methoxy-6-methylaminopyridine-3-carboxylic Acid (11). A mixture of **10** (0.95 g, 4.12 mmol), NaOH (0.18 g, 4.53 mmol), and 50% aqueous MeOH (20 mL) was heated to reflux for 1.5 h and cooled to room temperature. After evaporation of the volatiles, the aqueous solution was acidified with 35% aqueous HCl, and the resulting precipitate was collected by filtration, washed with water, and dried to give 0.81 g (91%) of **11**, mp 206−208 °C (EtOH). ^{1}H NMR (dimethyl sulfoxide-d_6) δ: 2.93 (d, 3H, J = 4.8), 3.89 (s, 3H), 7.25 (br. q, 1H, J = 4.8), 7.85 (s, 1H), 12.06 (s, 1H). MS *m*/*z*; 217 (MH^+). IR cm^{-1}; 3416, 1674, 1595, 1564, 1385, 1225. Anal. ($C_8H_9ClN_2O_3$) C, H, N, Cl.

Ethyl 2-Ethoxy-6-methylaminopyridine-3-carboxylate (13). A mixture of methyl 2-fluoro-6-methylaminopyridine-3-carboxylate (**12**, 4.5 g, 24.5 mmol), potassium *tert*-butoxide (5.48 g, 48.9 mmol), and EtOH (50 mL) was heated to reflux for 2 h and cooled to room temperature. After evaporation of

the solvent, saturated aqueous $NaHCO_3$ was added to the residue. The resultant solid was collected by filtration, washed successively with water and hexane, and dried to give 4.38 g (80%) of **13**, mp 94−96 °C (EtOH/diisopropyl ether). ^{1}H NMR δ: 1.34 (t, 3H, J = 7.0), 1.41 (t, 3H, J = 7.0), 2.95 (d, 3H, J = 5.0), 4.28 (q, 2H, J = 7.0), 4.42 (q, 2H, J = 7.0), 4.79 (br., 1H), 5.92 (d, 1H, J = 8.5), 8.00 (d, 1H, J = 8.5). MS m/z; 225 (MH^+). IR cm^{-1}; 3350, 1690, 1601, 1259, 1155. Anal. ($C_{11}H_{16}N_2O_3$) C, H, N.

Ethyl 5-Chloro-2-ethoxy-6-methylaminopyridine-3-carboxylate (14). In a similar manner to that described above, **14** was prepared from **13** and NCS in 90% yield, mp 126−127 °C (EtOH). ^{1}H NMR δ: 1.34 (t, 3H, J = 7.0), 1.44 (t, 3H, J = 7.0), 3.06 (d, 3H, J = 5.0), 4.28 (q, 2H, J = 7.0), 4.47 (q, 2H, J = 7.0), 5.29 (br., 1H), 8.00 (s, 1H). MS m/z; 259 (MH^+). IR cm^{-1}; 3389, 1709, 1593, 1570, 1227, 1080. Anal. ($C_{11}H_{15}ClN_2O_3$) C, H, N, Cl.

Ethyl 5-Bromo-2-ethoxy-6-methylaminopyridine-3-carboxylate (15). A solution of **13** (1.66 g, 7.41 mmol), *N*-bromosuccinimide (NBS, 1.32 g, 7.41 mmol), and DMF (15 mL) was heated at 80 °C for 1 h. The reaction mixture was poured into ice−water and the resulting precipitate was collected by filtration, washed successively with water and hexane, and dried to give 2.17 g (97%) of **15**, mp 126−127 °C (AcOEt/hexane). ^{1}H NMR δ: 1.35 (t, 3H, J = 7.0), 1.45 (t, 3H, J = 7.0), 3.06 (d, 3H, J = 5.0), 4.28 (q, 2H, J = 7.0), 4.48 (q, 2H, J = 7.0), 5.32 (br., 1H), 8.15 (s, 1H). MS m/z; 303 (MH^+). IR cm^{-1}; 3385, 1711, 1589, 1568, 1227. Anal. ($C_{11}H_{15}BrN_2O_3$) C, H, N, Br.

5-Chloro-2-ethoxy-6-methylaminopyridine-3-carboxylic Acid (16). In a similar manner to that described above, alkaline hydrolysis of **14** gave **16** in 81% yield, mp 161−162 °C (EtOH). ^{1}H NMR (dimethyl sulfoxide-d_6) δ: 1.32 (t, 3H, J = 7.0), 2.90 (d, 3H, J = 5.0), 4.38 (q, 2H, J = 7.0), 7.23 (br. q, 1H, J = 5.0), 7.85 (s, 1H), 12.00 (s, 1H). MS m/z; 231 (MH^+). IR cm^{-1}; 3348, 3323, 1713, 1603, 1450, 1385, 1331. Anal. ($C_9H_{11}ClN_2O_3$) C, H, N, Cl.

5-Bromo-2-ethoxy-6-methylaminopyridine-3-carboxylic Acid (17). In a similar manner to that described above, alkaline hydrolysis of **15** gave **17** in 96% yield, mp 169−170 °C (EtOH). ^{1}H NMR δ: 1.50 (t, 3H, J = 7.5), 3.08 (d, 3H, J = 5.0), 4.62 (q, 2H, J = 7.5), 5.56 (br., 1H), 8.30 (s, 1H), 10.33 (br. s, 1H). MS m/z; 275 (MH^+). IR cm^{-1}; 3348, 3321, 1707, 1599, 1450, 1381, 1329. Anal. ($C_9H_{11}BrN_2O_3$) C, H, N, Br.

Methyl 2-Chloro-6-methoxypyridine-3-carboxylate (20). A mixture of 2,6-dichloropyridine-3-carboxylic acid (**18**, 90%, 6.5 g, 30 mmol), potassium *tert*-butoxide (11.4 g, 0.10 mol), and MeOH (300 mL) was heated to reflux for 4 days and cooled to room temperature. After evaporation of the solvent, the residue was diluted with water and acidified with 35% aqueous HCl. The resulting solid was collected by filtration, washed with water, and dried to give 4.8 g (84%) of 2-chloro-6-methoxypyridine-3-carboxylic acid (**19**). A mixture of **19** (4.8 g, 25.6 mmol) and $SOCl_2$ (20 mL, 0.27 mol) was heated to reflux for 5 h and cooled to room temperature. Excess $SOCl_2$ was evaporated, and the residue was dissolved in toluene. After the volatiles were evaporated, the residue was redissolved in toluene. Again, the solution was concentrated to dryness, and the residue was dissolved in MeOH. The solution was heated to reflux for 1 h and cooled to room temperature. The solvent was evaporated, and the residue was diluted with water and extracted with $CHCl_3$. The solvent was evaporated and the residual solid was recrystallized from EtOH to afford 4.0 g (78%) of **20**, mp 71−72 °C. ^{1}H NMR δ: 3.92 (s, 3H), 4.00 (s, 3H), 6.70 (d, 1H, J = 8.8), 8.12 (d, 1H, J = 8.8). ^{13}C NMR δ: 52.40, 54.59, 109.22, 118.42, 142.96, 149.28, 164.75, 164.86. MS m/z; 202 (MH^+). IR cm^{-1}; 1736, 1597, 1491, 1321, 1246. Anal. ($C_8H_8ClNO_3$) C, H, N, Cl.

Methyl 6-Methoxy-2-methylaminopyridine-3-carboxylate (21). A solution of **20** (2.6 g, 12.9 mmol) and 30% $MeNH_2$ in EtOH (5.5 g, 53.2 mmol) in EtOH (20 mL) was heated to reflux for 12 h and cooled to room temperature. After evaporation of the volatiles, the residue was dissolved in $CHCl_3$. The solution was washed successively with water and brine and dried over anhydrous $MgSO_4$. The solvent was evaporated, and the residue was chromatographed on silica gel with hexane/AcOEt = 10/1 to give 2.1 g (83%) of **21** as a solid, mp 50−52 °C (AcOEt/hexane). ^{1}H NMR δ: 3.05 (d, 3H, J = 5.0), 3.81 (s, 3H), 3.94 (s, 3H), 5.92 (d, 1H, J = 8.4), 7.97 (d, 1H, J = 8.4), 8.00 (br., 1H). MS m/z; 197 (MH^+). IR cm^{-1}; 3377, 2947, 1686, 1593, 1251, 1232. Anal. ($C_9H_{12}N_2O_3$) C, H, N.

Methyl 5-Chloro-6-methoxy-5-methylaminopyridine-3-carboxylate (22). In a similar manner to that described above, **22** was prepared from **21** and NCS in 85% yield, mp 120−122 °C (AcOEt/hexane). ^{1}H NMR δ: 3.04 (d, 3H, J = 5.0), 3.82 (s, 3H), 4.03 (s, 3H), 7.95 (br., 1H), 8.01 (s, 1H). MS m/z; 231 (MH^+). IR cm^{-1}; 3363, 1678, 1600, 1589, 1231. Anal. ($C_9H_{11}ClN_2O_3$) C, H, N, Cl.

5-Chloro-6-methoxy-2-methylaminopyridine-3-carboxylic Acid (23). In a similar manner to that described above, alkaline hydrolysis of **22** gave **23** in 84% yield. Analysis sample of **23** was obtained by recrystallization from EtOH. The solid was sublimated at 136 °C. ^{1}H NMR δ: 3.06 (d, 3H, J = 4.8), 4.05 (s, 3H), 7.86 (br., 1H), 8.05 (s, 1H). MS m/z; 217 (MH^+). IR cm^{-1}; 3389, 1670, 1582, 1259, 1238. Anal. ($C_8H_9ClN_2O_3$) C, H, N, Cl.

Methyl 6-Ethylamino-2-fluoropyridine-3-carboxylate (25). Et_3N (10.5 g, 104 mmol) was added to a solution of methyl 2,6-difluoropyridine-3-carboxylate (**24**, 9.0 g, 52 mmol) and $EtNH_2$·HCl (4.24 g, 52 mmol) in DMF (50 mL) under ice-cooling. The mixture was stirred at the same temperature for 1 h. The solvent was evaporated, and the residue was dissolved in AcOEt. The solution was washed successively with water and brine and dried over anhydrous $MgSO_4$. The solvent was evaporated, and the residue was chromatographed on silica gel with $CHCl_3$/hexane = 1/1 to $CHCl_3$ to give first 3.2 g (31%) of methyl 2-ethylamino-6-fluoropyridine-3-carboxylate (**26**) and then 6.3 g (61%) of **25** both as solids. **25**; mp 116−117 °C (AcOEt/hexane). ^{1}H NMR δ: 1.27 (t, 3H, J = 7.5), 3.3−3.47 (m, 2H), 3.87 (s, 3H), 5.12 (br., 1H), 6.22 (dd, 1H, J = 2.0, 8.5), 8.07 (dd, 1H, J = 8.5, 10.0). MS m/z; 199 (MH^+). IR cm^{-1}; 3267, 3132, 1701, 1626, 1437, 1298, 1151. Anal. ($C_9H_{11}FN_2O_2$) C, H, N, F.

26; mp 127−128 °C (hexane/AcOEt). ^{1}H NMR δ: 1.26 (t, 3H, J = 7.5), 3.42−3.6 (m, 2H), 3.86 (s, 3H), 6.05 (dd, 1H, J = 3.0, 8.0), 8.17 (dd, 1H, J = 8.0, 8.0). MS m/z; 199 (MH^+). IR cm^{-1}; 3350, 1701, 1612, 1583, 1516, 1437, 1294, 1259, 1130. Anal. ($C_9H_{11}FN_2O_2$) C, H, N, F.

Methyl 6-Dimethylamino-2-fluoropyridine-3-carboxylate (27) and Methyl 2-Dimethylamino-6-fluoropyridine-3-carboxylate (28). A mixture of **24** (10.0 g, 58 mmol), Me_2NH (11.4 g, 127 mmol), and EtOH (50 mL) was stirred at −20 °C for 4 h. The reaction mixture was then diluted with water, extracted with AcOEt/hexane = 1/1, and washed with brine. The solvent was evaporated, and the residue was chromatographed on silica gel with $CHCl_3$/hexane = 10/1 to give 7.9 g (69%) of a mixture of **27** and **28** as a solid. ^{1}H NMR δ: 3.02 (s, 1.2H, Me_2M of **28**), 3.15 (s, 6H, Me_2M of **27**), 3.87 (s, 3H, CO_2Me of **27**), 3.99 (s, 0.6H, CO_2Me of **28**), 6.18 (dd, 0.2H, J = 3.5, 8.5, pyridine-5H of **28**), 6.31 (dd, 1H, J = 2.5, 8.8, pyridine-5H of **27**), 8.02 (dd, 0.2H, J = 8.5, 8.5, pyridine-4H of **28**), 8.08 (dd, 1H, J = 8.8, 10.0, pyridine-4H of **27**). MS m/z; 199 (MH^+). The mixture (1.35 g) was recrystallized from AcOEt/hexane to afford 0.3 g of **27**, mp 69−70 °C. IR cm^{-1}; 1728, 1622, 1537, 1290. Anal. ($C_9H_{11}FN_2O_2$) C, H, N, F.

Methyl 6-Ethylamino-2-methoxypyridine-3-carboxylate (29). In a similar manner to that described above, **29** was prepared from **25** in 82% yield, mp 113−115 °C (AcOEt/hexane). ^{1}H NMR (dimethyl sulfoxide-d_6) δ: 1.15 (t, 3H, J = 7.0), 3.23−3.42 (m, 2H), 3.67 (s, 3H), 3.84 (s, 3H), 6.04 (d, 1H, J = 9.0), 7.39 (br., 1H), 7.79 (d, 1H, J = 9.0). MS m/z; 211 (MH^+). IR cm^{-1}; 3366, 1693, 1596, 1578, 1258. Anal. ($C_{10}H_{14}N_2O_3$) C, H, N.

Methyl 6-Dimethylamino-2-methoxypyridine-3-carboxylate (30). A mixture of nonseparated **27** and **28** (6.55 g, 35 mmol), potassium *tert*-butoxide (9.26 g, 83 mmol), and MeOH (150 mL) was heated to reflux for 2 h and cooled to room temperature. After evaporation of the solvent, saturated

aqueous $NaHCO_3$ was added to the residue. The resultant solid was collected by filtration, washed successively with water and hexane, and dried to give 4.85 g (70%) of **30**, mp 102−105 °C (AcOEt/hexane). ^{1}H NMR δ: 3.12 (s, 6H), 3.82 (s, 3H), 3.98 (s, 3H), 6.04 (d, 1H, J = 8.8), 8.02 (d, 1H, J = 8.8). MS m/z; 211 (MH^+). IR cm^{-1}; 2873, 1709, 1605, 1558, 1387, 1250, 1171. Anal. ($C_{10}H_{14}N_2O_3$) C, H, N.

Methyl 5-Chloro-6-ethylamino-2-methoxypyridine-3-carboxylate (32). In a similar manner to that described above, **32** was prepared from **29** and NCS in 91% yield, mp 96−97 °C (AcOEt). ^{1}H NMR δ: 1.28 (t, 3H, J = 7.0), 3.43−3.65 (m, 2H), 3.81 (s, 3H), 3.99 (s, 3H), 5.32 (br., 1H), 8.01 (s, 1H). MS m/z; 245 (MH^+). IR cm^{-1}; 3368, 1684, 1598, 1578. Anal. ($C_{10}H_{13}ClN_2O_3$) C, H, N, Cl.

Methyl 5-Bromo-6-ethylamino-2-methoxypyridine-3-carboxylate (33). In a similar manner to that described above, **33** was prepared from **29** and NBS in 91% yield, mp 104−105 °C (AcOEt/hexane). ^{1}H NMR δ: 1.28 (t, 3H, J = 7.0), 3.44−3.65 (m, 2H), 3.83 (s, 3H), 4.00 (s, 3H), 5.35 (br., 1H), 8.16 (s, 1H). MS m/z; 289 (MH^+). IR cm^{-1}; 3360, 1712, 1599, 1589, 1566, 1234. Anal. ($C_{10}H_{13}BrN_2O_3$) C, H, N, Br.

Methyl 5-Bromo-6-dimethylamino-2-methoxypyridine-3-carboxylate (34). In a similar manner to that described above, **34** was prepared from **30** and NBS in 63% yield, mp 70−71 °C (hexane). ^{1}H NMR δ: 3.18 (s, 6H), 3.82 (s, 3H), 3.98 (s, 3H), 8.22 (s, 1H). MS m/z; 289 (MH^+). IR cm^{-1}; 2947, 1683, 1597, 1393, 1354. Anal. ($C_{10}H_{13}BrN_2O_3$) C, H, N, Br.

5-Chloro-6-ethylamino-2-methoxypyridine-3-carboxylic Acid (35). In a similar manner to that described above, alkaline hydrolysis of **32** gave **35** in 96% yield, mp 143−145 °C (EtOH). ^{1}H NMR (dimethyl sulfoxide-d_6) δ:0.17 (t, 3H, J = 7.5), 3.37−3.55 (m, 2H), 3.87 (s, 3H), 7.30 (br. t, 1H, J = 5.0), 7.86 (s, 1H), 12.06 (s, 1H). MS m/z; 231 (MH^+). IR cm^{-1}; 3414, 1680, 1595, 1560, 1381, 1225. Anal. ($C_9H_{11}ClN_2O_3$) C, H, N, Cl.

5-Bromo-6-ethylamino-2-methoxypyridine-3-carboxylic Acid (36). In a similar manner to that described above, alkaline hydrolysis of **33** gave **36** in 95% yield, mp 169−170 °C (^{i}PrOH/EtOH). ^{1}H NMR δ: 1.31 (t, 3H, J = 7.5), 3.47−3.65 (m, 2H), 4.12 (s, 3H), 5.64 (br., 1H), 829 (s, 1H), 10.16 (br. s, 1H). MS m/z; 275 (MH^+). IR cm^{-1}; 3429, 3408, 1672, 1590, 1570, 1379, 1284, 1227. Anal. ($C_9H_{11}BrN_2O_3$) C, H, N, Br.

5-Bromo-6-dimethylamino-2-methoxypyridine-3-carboxylic Acid (37). In a similar manner to that described above, alkaline hydrolysis of **34** gave **37** in 78% yield, mp 195−196 °C (AcOEt). ^{1}H NMR (dimethyl sulfoxide-d_6) δ: 3.12 (s, 6H), 3.87 (s, 3H), 8.08 (s, 1H), 12.42 (s, 1H). MS m/z; 275 (MH^+). IR cm^{-1}; 1653, 1591, 1398, 1276, 1246. Anal. ($C_9H_{11}BrN_2O_3$) C, H, N, Br.

General Procedure for the Preparation of the Carboxamide Derivatives [49−56, 58−67, (*R*)-50 and (*S*)-50, (*R*)-53 and (*S*)-53, (*R*)-57, and (*R*)-68]. A mixture of the appropriate carboxylic acid (10 mmol), amine (11 mmol), EDC (12 mmol), and CH_2Cl_2 (80 mL) was stirred at room temperature for 4−5 h. The reaction mixture was washed successively with H_2O, 10% aqueous NaOH, and brine. The solvent was evaporated, and the residue was chromatographed on silica gel. The free base thus obtained was either recrystallized from the solvents shown in Tables 2−4 or converted into a fumarate or oxalate in a usual manner, and then recrystallized from the solvents shown in Tables 2−4.

Binding Assays for Dopamine D$_2$ and Serotonin 5-HT$_3$ Receptors. The binding assays were carried out according to the method described in the previous papers.[24,33]

Effect on Apomorphine-Induced Emesis in Dogs.[34] Male beagle dogs, weighing 10−16 kg, were used. Groups of three to six dogs received a subcutaneous injection of apomorphine hydrochloride (0.3 mg/kg) 2 h after pretreatment with test compounds. The frequency of emesis was then counted for 1 h.

Effects on Cisplatin and Morphine-Induced Emesis in Ferrets and Dogs. Ferrets (n = 5) were used to investigate the antiemetic effects of (*R*)-**53**, metoclopramide, and ondansetron against emesis induced by cisplatin, and dogs (n = 5) were used in morphine-induced emetic responses. Although both ferrets and dogs exhibited emetic responses, dogs were more sensitive to morphine than were ferrets to cisplatin.[58] Each animal received either (*R*)-**53**, haloperidol, metoclopramide, ondansetron, or saline intravenously 15 min before morphine injection (3 mg/kg, sc). In the case of cisplatin-induced emetic responses, each ferret simultaneously received (*R*)-**53**, metoclopramide, or ondansetron (iv) and cisplatin (10 mg/kg, iv). To evaluate the activity of (*R*)-**53**, (haloperidol), metoclopramide, and ondansetron given orally, each animal received test compounds or vehicle 60 min before administration of morphine (3 mg/kg, sc) or 30 min before cisplatin (10 mg/kg, iv) administration. The latency to first retch and vomit and the number of vomits were recorded for each animal for 4 h (cisplatin-induced emesis) or 30 min (morphine-induced emesis). Vomiting was scored as oral expulsion of liquid or solid stomach content. The doses of test compounds and inhibition percentage are shown in Figures 1 and 2. Significant differences were evaluated using nonparametric Dunnett's multiple comparison test or the Wilcoxon rank sum test. The significance level was set at $p < 0.05$, $p < 0.01$, and $p < 0.001$. The ID_{50} values of test compounds (dose causing 50% inhibition of the number of emetic episodes elicited by various test compounds) were determined by the method of logit analysis.

X-ray Crystallographic Analysis of (*R*)-53. Suitable crystals of (*R*)-**53** were grown from EtOH solutions. Crystal data: A colorless crystal of $C_{24}H_{34}BrN_5O_{10}$ having approximate dimensions of 0.3 × 0.5 × 1.0 mm; FW = 632.46; orthorhombic; space group $P2_12_12$; a = 12.103(5) Å; b = 27.781(6) Å; c = 17.272(5) Å; V = 5807(2) Å^3; Z = 8; D_{calcd} = 1.450 g/cm^3; $F(000)$ = 2624; μ(CuKα) = 24.67 cm^{-1}; T = 293 K; $\alpha\Delta_{max}$ = −0.40; $\Delta\rho_{max}$ = 0.56e/Å^3; GOF = 1.89 for 737 parameters.

All measurements were made on a Rigaku AFC5R diffractometer with graphite monochromated CuKα radiation (λ = 1.54178 Å). The 5490 reflections were collected using the ω−2θ scan technique. The intensities of three representative reflections were measured after every 100 reflections. No decay correction was applied. The data were corrected for Lorentz and polarization effects. A correction for secondary extinction was applied. The structure was solved by direct methods (SIR92) and expanded using Fourier technique (DIRDIF 94). Non-hydrogen atoms were refined with anisotropic temperature factors. All hydrogen atoms were included at idealized positions but not refined. The final cycle of full-matrix least-squares refinement (SHELXL-93) was based on 4575 observed reflections and 737 variable parameters with $1 > 2\sigma(I)$ and converged at R = 0.057 and R_W = 0.229.

All the calculations were performed using the teXsan (Molecular Structure Co.). A refinement of the Flack's χ parameter was carried out to determine at the absolute configuration. The value of refined χ−0.01(3) indicated the correct absolute stereochemistry.

Acknowledgment. We thank Prof. T. Ishida and Ms. Y. In of Osaka University of Pharmaceutical Sciences for X-ray crystallographic analysis.

Supporting Information Available: Crystallographic details for (*R*)-**53** including tables of atomic coordinates, thermal parameters, bond angles, and bond lengths; tables of biological data for (*R*)-**53**, metoclopramide, and ondansetron. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM020270N