Synthesis and Structure–Activity Relationships of 2,3-Dihydrobenzofuran-7-carboxamide Derivatives as Potent Serotonin-3 (5-HT_3) Receptor Antagonists

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A series of *N*-(azabicyclo-3-yl)-2,3-dihydrobenzofuran-7-carboxamide derivatives were synthesized and evaluated for serotonin-3 (5-HT_3) receptor antagonistic activities assessed by 5-HT_3 receptor binding (*in vitro*) and by the ability to antagonize the von Bezold–Jarisch reflex in rats (*in vivo*). In these compounds, 1-azabicyclo[2.2.2]oct-3-yl derivatives were more potent than 8-methyl-8-azabicyclo[3.2.1]oct-3-yl derivatives for 5-HT_3 receptor antagonistic activities. The introduction of methyl groups at position 2 of the dihydrobenzofuran ring increased the pharmacological activities (dimethyl > monomethyl > dihydro). Furthermore, the stereoisomers of dimethyl-, monomethyl-, and dihydrobenzofuran derivatives were prepared to evaluate the stereoselectivity of their 5-HT_3 receptor binding affinities. Concerning the basic part, the compounds bearing (*S*)-1-azabicyclo[2.2.2]octan-3-yl moiety were more potent than their counterparts. With respect to the methyl substituent at position 2 of the dihydrobenzofuran ring, the rank order of the potency was dimethyl ≧ (2*S*)-methyl > (2*R*)-methyl > dihydro. These results suggest that the (2*S*)-methyl group of the dihydrobenzofuran part contributes to the enhancement of the pharmacological activity. Among these compounds, (*S*)-*N*-(1-azabicyclo[2.2.2]oct-3-yl)-5-chloro-2,3-dihydro-2,2-dimethylbenzofuran-7-carboxamide hydrochloride (24) showed the highest affinity for 5-HT_3 receptors (K_i = 0.055 nM), and the most potent antagonistic activity on the von Bezold–Jarisch reflex (ED_{50} = 0.18 μg/kg i.v.).

Keywords benzofurancarboxamide; 5-HT_3 receptor antagonist; structure–activity relationship; 5-HT_3 receptor binding; von Bezold–Jarisch reflex; partition coefficient

It has been postulated that there are multiple subtypes for the serotonin (5-HT) receptor in brain and peripheral tissues, including 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, 5-HT_{1D}, 5-HT_2, 5-HT_3 and 5-HT_4.[1a–c] The discovery of these subtypes stimulated subsequent research to design pharmacological agents selective for each of the 5-HT receptor subtypes. Recently, a number of 5-HT_3 receptor antagonists such as granisetron (**1**) and zacopride (**2**) have been reported.[2a,b] These compounds have been shown to be clinically effective in the treatment of chemotherapy-induced emesis.[3a,b] Antagonism of 5-HT_3 receptors has also been implicated for the treatment of migraine, dementia, anxiety, schizophrenia, and drug abuse.[4a,e]

In the previous paper, we reported that *N*-(1-azabicyclo-[2.2.2]oct-3-yl)-6-chloro-3,4-dihydro-4-methyl-3-oxo-2*H*-1,4-benzoxazine-8-carboxamide (**3**, Y-25130, Fig. 1) was a potent and selective 5-HT_3 receptor antagonist.[5] From the structure–activity relationships (SAR) of its derivatives, the compounds bearing 1-azabicyclo[2.2.2]oct-3-yl moiety as the basic part exhibited more potent antagonistic activity than those bearing the other basic moieties, *i.e.*, 8-methyl-8-azabicyclo[3.2.1]oct-3-yl, 1-benzyl-4-piperidinyl, and 2-(*N*,*N*-diethylamino)ethyl derivatives. We also described that *N*-[(1-butyl-2-pyrrolidinyl)methyl]-2,3-dihydro-5-sulfamoylbenzofuran-7-carboxamide (**4**, Fig. 1) was a potent dopamine receptor antagonist to exhibit an atypical antipsychotic profile.[6] These results prompted us to reinvestigate the replacement of the 3,4-dihydro-3-oxo-2*H*-1,4-benzoxazine-8-carboxamide moiety with the 2,3-dihydrobenzofuran-7-carboxamide moiety. We expected that the *N*-(1-azabicyclo[2.2.2]oct-3-yl)-2,3-dihydrobenzofuran-7-carboxamides would exert potent and selective 5-HT_3 receptor antagonistic activities. Some of them showed potent antagonistic activity on von Bezold–Jarisch (BJ) reflux and high affinity for 5-HT_3 receptors, although none of the synthesized 2,3-dihydrobenzofuran-7-carboxamides showed any antidopaminergic activity. The present paper describes the synthesis and SAR of 2,3-dihydrobenzofuran-7-carboxamides and discusses the stereospecificity for the 5-HT_3 receptor affinity of some of them.

Chemistry 2,3-Dihydrobenzofuran-7-carboxamides **6**, **7** and **10**—**17**, listed in Table I, were prepared from the corresponding carboxylic acids by coupling with appropriate amines *via* mixed anhydrides or acid chlorides (Chart 1). The ester **8** and **9** were prepared by reaction of the

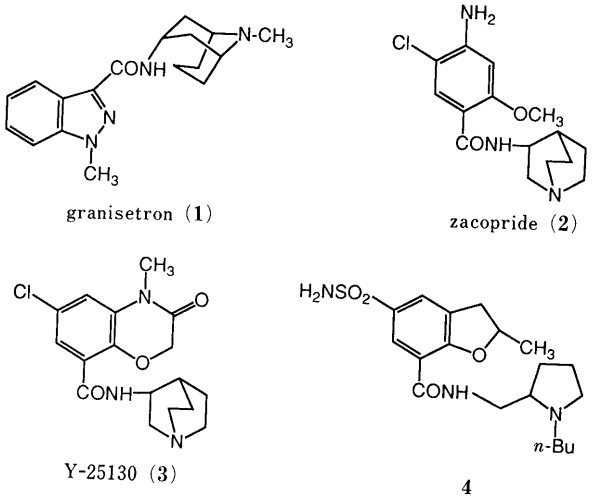
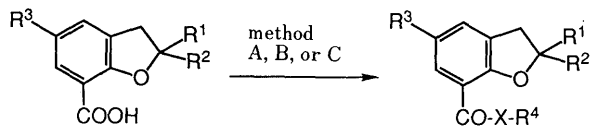


Fig. 1

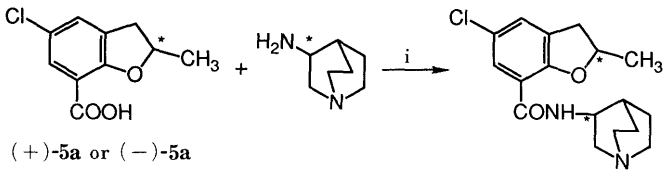


5a–i 6–17, 22–25

	R^1	R^2	R^3
5a	H	CH_3	Cl
5b	H	CH_3	Br
5c	H	CH_3	F
5d	H	CH_3	H
5e	H	CH_3	SO_2NHCH_3
5f	H	CH_3	SO_2CH_3
5g	H	CH_3	SCH_3
5h	H	H	Cl
5i	CH3	CH_3	Cl

reagents : method A i) pivaloyl chloride, NEt_3 ii) R^4-NH_2
method B i) $SOCl_2$ ii) R^4-NH_2
method C i) $SOCl_2$ ii) R^4-OH, BuLi

Chart 1



(+)-5a or (−)-5a 18–21

reagents : i) pivaloyl chloride, NEt_3

Chart 2

corresponding acid chlorides with lithium alkoxides in tetrahydrofuran (THF). The 2,3-dihydrobenzofuran-7-carboxylic acids (**5a—i**) were prepared by the published procedures.[6,7] (±)-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 in accordance with the reported procedure.[8] The compounds **6**, **7** and **10—15** were obtained as diastereomeric mixtures because both of the starting materials were racemates. Compounds **16** and **17** were racemates. The diastereomeric ratios of some

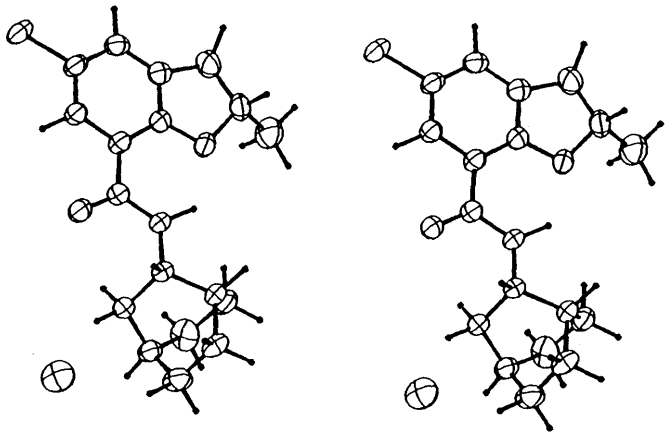
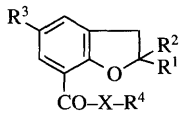


Fig. 2. ORTEP Drawing of **20**

TABLE I. Physical Data of 2,3-Dihydrobenzofuran-7-carboxamides **6—17**



Compd. No.	R^1	R^2	R^3	X	$R^{4a)}$	Yield (%) Method	mp (°C) Solvent[b)]	Formula	Analysis (%) Calcd (Found) C	H	N
6	CH_3	H	Cl	NH	Q	48.6 B	267—269 EtOH–IPE	$C_{17}H_{21}ClN_2O_2\cdot$ HCl	57.15 (56.95	6.21 6.28	7.84 7.83)
7	CH_3	H	Cl	NH	T	4.5 B	243—245 EtOH–IPE	$C_{18}H_{23}ClN_2O_2\cdot$ $HCl\cdot 2/3H_2O$	56.40 (56.40	6.66 6.50	7.30 7.36)
8	CH_3	H	Cl	O	Q	49.8 C	246—248 EtOH–IPE	$C_{17}H_{20}ClNO_3\cdot$ HCl	56.99 (56.74	5.91 5.92	3.91 3.93)
9	CH_3	H	Cl	O	T	19.2 C	160—162 EtOH	$C_{18}H_{22}ClNO_3\cdot$ $1/2fumarate\cdot H_2O$	58.32 (58.06	6.36 6.17	3.40 3.04)
10	CH_3	H	Br	NH	Q	29.2 A	285—290 (dec.) EtOH–IPE	$C_{17}H_{21}BrN_2O_2\cdot$ $HCl\cdot 1/4H_2O$	50.26 (50.21	5.58 5.45	6.90 6.86)
11	CH_3	H	F	NH	Q	40.8 A	222—225 (dec.) EtOH–IPE	$C_{17}H_{21}FN_2O_2\cdot$ $HCl\cdot H_2O$	56.90 (56.69	6.74 6.58	7.81 7.83)
12	CH_3	H	H	NH	Q	51.3 A	232—233 EtOH	$C_{17}H_{22}N_2O_2$	63.24 (62.99	7.18 7.26	8.68 8.66)
13	CH_3	H	SO_2NHCH_3	NH	Q	47.6 A	258—260 EtOH–IPE	$C_{18}H_{25}N_3O_4S\cdot$ $HCl\cdot 1/2H_2O$	50.88 (50.61	6.41 6.23	9.89 9.79)
14	CH_3	H	SO_2CH_3	NH	Q	11.4 A	256—258 EtOH–IPE	$C_{18}H_{24}N_2O_4S\cdot$ $HCl\cdot 1/2H_2O$	52.74 (52.90	6.39 6.27	6.83 6.83)
15	CH_3	H	SCH_3	NH	Q	37.6 A	186—187 EtOH	$C_{18}H_{24}N_2O_2S\cdot$ fumarate	58.91 (58.80	6.29 6.31	6.25 6.29)
16	H	H	Cl	NH	Q	49.6 A	269—272 EtOH–IPE	$C_{16}H_{19}ClN_2O_2\cdot$ $HCl\cdot H_2O$	53.20 (53.07	6.14 6.01	7.75 7.81)
17	CH_3	CH_3	Cl	NH	Q	28.2 A	287—290 (dec.) EtOH–IPE	$C_{18}H_{23}ClN_2O_2\cdot$ HCl	58.16 (58.26	6.46 6.45	7.58 7.50)

a) Q, (±)-1-azabicyclo[2.2.2]oct-3-yl. T, *endo*-8-methyl-8-azabicyclo[3.2.1]oct-3-yl. *b*) IPE, isopropyl ether.

TABLE II. Spectral Data of 2,3-Dihydrobenzofuran-7-carboxamides **6**—**17**

Compd. No.	IR (C=O) $\nu_{cm^{-1}}^{KBr}$	^{1}H-NMR	
		Solvent	δ (J=Hz)
6	1670	$CDCl_3$	1.56 (3H, d, J=7), 1.72—2.26 (4H, m), 2.26—2.56 (1H, m), 2.86 (1H, dd, J=7, 7), 3.04—3.92 (6H, m), 3.48 (1H, dd, J=7, 7), 4.32—4.72 (1H, m), 5.20 (1H, dq, J=7, 7), 7.24 (1H, d, J=2), 7.78 (1H, d, J=2), 7.80—8.08 (1H, br s)
7	1660	$CDCl_3$	1.56 (3H, d, J=7), 1.78—2.56 (8H, m), 2.82 (3H, s), 2.88 (1H, dd, J=7, 7), 3.40 (1H, dd, J=7, 7), 3.68—4.04 (2H, m), 4.22—4.56 (1H, m), 4.92—5.36 (1H, m), 7.24 (1H, d, J=2), 7.80 (1H, d, J=2), 7.84—8.14 (1H, br s)
8	1705	$CDCl_3$	1.52 (3H, d, J=7), 1.72—2.22 (4H, m), 2.38—2.68 (1H, m), 2.84 (1H, dd, J=7, 7), 3.12—3.64 (6H, m), 3.78 (1H, dd, J=7, 7), 5.10 (1H, dq, J=7, 7), 5.22—5.48 (1H, m), 7.32 (1H, d, J=2), 7.62 (1H, d, J=2)
9	1720	CF_3COOH	1.56 (3H, d, J=7), 2.12—2.80 (8H, m), 2.88 (1H, dd, J=7, 7), 3.02 (3H, s), 3.42 (1H, dd, J=7, 7), 4.04—4.32 (2H, m), 5.00—5.38 (1H, m), 5.42—5.78 (1H, m), 7.38 (1H, d, J=2), 7.66 (1H, d, J=2)
10	1665	$CDCl_3$	1.56 (3H, d, J=7), 1.82—2.24 (4H, m), 2.24—2.56 (1H, m), 2.88 (1H, dd, J=7, 7), 3.08—3.52 (5H, m), 3.34 (1H, dd, J=7, 7), 3.52—3.84 (1H, m), 4.28—4.68 (1H, m), 5.14 (1H, dq, J=7, 7), 7.40 (1H, d, J=2), 7.88 (1H, d, J=2), 7.86—8.08 (1H, br s)
11	1660	CF_3COOH	1.64 (3H, d, J=7), 2.12—2.48 (4H, m), 2.48—2.68 (1H, m), 2.98 (1H, dd, J=7, 7), 3.48 (1H, dd, J=7, 7), 3.50—3.84 (5H, m), 3.92—4.36 (1H, m), 4.60—4.92 (1H, m), 5.16—5.52 (1H, m), 7.20 (1H, dd, J=2, 10), 7.48 (1H, dd, J=2, 10), 8.90—9.14 (1H, br s)
12	1665	CF_3COOH	1.64 (3H, d, J=7), 2.16—2.52 (4H, m), 2.52—2.76 (1H, m), 3.00 (1H, dd, J=7, 7), 3.52 (1H, dd, J=7, 7), 3.44—3.88 (5H, m), 3.96—4.32 (1H, m), 4.68—4.98 (1H, m), 5.16—5.50 (1H, m), 7.08 (1H, dd, J=6, 6), 7.52 (1H, d, J=6), 7.80 (1H, d, J=6), 9.04—9.32 (1H, br s)
13	1660	CF_3COOH	1.68 (3H, d, J=7), 2.12—2.48 (4H, m), 2.48—2.68 (1H, m), 2.78 (3H, s), 3.08 (1H, dd, J=7, 7), 3.38—3.84 (6H, m), 3.92—4.32 (1H, m), 4.62—4.96 (1H, m), 4.62—4.96 (1H, m), 7.64—8.08 (1H, br s), 7.98 (1H, d, J=2), 8.22 (1H, d, J=2), 8.60—8.84 (1H, br s)
14	1660	CF_3COOH	1.66 (3H, d, J=7), 2.12—2.48 (4H, m), 2.48—2.72 (1H, m), 3.12 (1H, dd, J=7, 7), 3.36 (3H, s), 3.44—3.94 (6H, m), 3.92—4.34 (1H, m), 4.60—4.96 (1H, m), 5.28—5.72 (1H, m), 8.02 (1H, d, J=2), 8.50 (1H, d, J=2), 8.58—8.78 (1H, br s)
15	1655	DMSO-d_6	1.46 (3H, d, J=7), 1.58—1.98 (4H, m), 1.98—2.20 (1H, m), 2.44 (3H, s), 2.76 (1H, dd, J=7, 7), 2.90—3.22 (6H, m), 3.68 (1H, dd, J=7, 7), 4.04—4.38 (1H, m), 5.08 (1H, dq, J=7, 7), 7.34 (1H, d, J=2), 7.44 (1H, d, J=2), 7.96—8.16 (1H, br s)
16	1660	CF_3COOH	2.06—2.44 (4H, m), 2.44—2.66 (1H, m), 3.56 (2H, t, J=8), 3.40—3.88 (5H, m), 3.88—4.38 (1H, m), 4.60—4.84 (1H, m), 4.90 (2H, t, J=8), 7.42 (1H, d, J=2), 7.72 (1H, d, J=2), 8.68—8.92 (1H, br s)
17	1660	DMSO-d_6	1.48 (3H, d, J=7), 1.50 (3H, d, J=7), 1.68—2.04 (4H, m), 2.04—2.24 (1H, m), 3.08 (2H, s), 3.10—3.36 (5H, m), 3.36—3.76 (1H, m), 4.12—4.24 (1H, m), 7.38 (1H, d, J=2), 7.46 (1H, d, J=2), 7.96—8.12 (1H, br s)

compounds, which were recrystallized, were determined to be within a range of 0.9 to 1.1 by high performance liquid chromatography (HPLC).

The stereoisomers (**18**—**21**) of the 2,3-dihydrobenzofuran-7-carboxamide **6** shown in Table IV were prepared by coupling the enantiomers of 5-chloro-2-methyl-2,3-dihydrobenzofuran-7-carboxylic acid with those of 3-amino-1-azabicyclo[2.2.2]octane.[9] The reaction was performed by the mixed anhydride method (Chart 2). The starting enantiomers of 2,3-dihydrobenzofuran-7-carboxylic acid ((−)-**5a** and (+)-**5a**) were obtained by fractional crystallization of their cinchonidine and cinchonine salts, respectively. The coupling of (−)-**5a** with (*R*)-(+)-3-amino-1-azabicyclo[2.2.2]octane yielded compound **20**. The configuration at position 2 of the dihydrobenzofuran ring of compound **20** was determined as *S* by X-ray crystallography (Fig. 2).[10] With this information, we were then able to assign the absolute configuration of the other three isomers (**18**, **19**, **21**) as shown in Table IV. The enantiomers (**22**—**25**) of compounds **16** and **17** listed in Table IV were synthesized in a manner similar to that in Chart 2 from the corresponding carboxylic acids and enantiomers of 3-amino-1-azabicyclo[2.2.2]octane.

Pharmacological Results and Discussion

Compounds **6**—**17** were evaluated for 5-HT_3 receptor antagonistic activities *in vitro* by [^{3}H]granisetron binding[11] and *in vivo* by their ability to antagonize the 5-HT induced bradycardia (BJ reflex) in rats.[12] The results of these derivatives are presented in Tables III and IV.

The importance of constraining the basic nitrogen within an azabicyclic system was demonstrated in the previous paper.[5] Furthermore, the 1-azabicyclo[2.2.2]oct-3-yl moiety was preferable to the 8-methyl-8-azabicyclo[3.2.1]-oct-3-yl moiety in 3,4-dihydro-3-oxo-2*H*-1,4-benzoxazine-8-carboxamide for the 5-HT_3 receptor antagonistic activities.[5] We observed the same results in this series. Thus compounds **6** and **8** bearing the 1-azabicyclo-[2.2.2]oct-3-yl moiety were more potent than **7** and **9** bearing the 8-methyl-8-azabicyclo[3.2.1]oct-3-yl moiety (Table III).

Compounds **8** and **9** bearing an ester linkage at position 7 of the dihydrobenzofuran ring showed less potent activity than the corresponding carboxamide derivatives **6** and **7**, respectively (Table III). Interestingly, the X-ray crystallographic analysis of **20** (Fig. 2) showed that there exists an intramolecular hydrogen bond between the amide-hydrogen and the oxygen atom of the furan ring, and that the amide is coplanar to the furan ring. The replacement of the carboxamide linkage with the ester reduced potency, suggesting that the conformational restraint imposed by the intramolecular hydrogen bond is important to increase

the activity.

Replacement of the Cl atom at position 5 with Br, F, or H atom (**10**, **11**, **12**, respectively) resulted in reduced activity. Compounds **13**, **14** and **15**, bearing $MeNHSO_2$, $MeSO_2$, and MeS groups, respectively, at the same position were virtually inactive (Table III). The presence of small substituents (*e.g.*, F, Cl, Br) at the 5 position of the benzofuran ring may be tolerated, but larger substituents (*e.g.*, $MeNHSO_2$, $MeSO_2$) resulted in a marked reduction in potency.

The introduction of methyl groups at position 2 of the dihydrobenzofuran ring increased the pharmacological activity (dimethyl **17** > monomethyl **6** > dihydro **16**).

TABLE III. 5-HT_3 Receptor Antagonistic Activities of 2,3-Dihydrobenzofuran-7-carboxamides **6**—**17**

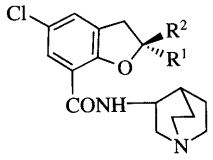
Compd. No.	[^{3}H]Granisetron binding K_i (nM)	BJ reflex[a] % inhibition ± S.E.M. (μg/kg i.v.)		
		0.5	5	50
6	0.26	91 ± 8.0	89 ± 8.0	100 ± 0
7	1.1	17 ± 3.3	97 ± 3.0	97 ± 2.5
8	2.9		8 ± 8.6	99 ± 0.5
9	9.6		12 ± 5.0	95 ± 4.5
10	0.47	32 ± 22.5		
11	0.7	23 ± 16.6		
12	4.8	28 ± 14.0	89 ± 7.0	
13	> 1000	6 ± 12.0		
14	> 1000	0 ± 0	38 ± 41.5	23 ± 27.7
15	47	0 ± 0		
16	0.47	49 ± 21.6	95 ± 4.5	
17	0.11	89 ± 11.5		

a) Serotonin was administered at a dose of 10 μg/kg i.v. 5 min posttreatment with drug at the specified dose.

A number of biologically active asymmetric compounds have been shown to have a clear stereoselectivity for binding to receptor or to an enzyme molecule. Compounds **6**, **16** and **17** were able to be composed of two or four stereoisomers. Therefore we investigated the SAR of their stereoisomers in detail by 5-HT_3 receptor binding affinity assay (*in vitro*). Among four stereoisomers of the 2-methyl-substituted compound **6**, compounds **18** and **20**, with the 3*S* configuration at the 1-azabicyclo[2.2.2]oct-3-yl moiety showed higher affinity for the 5-HT_3 receptors than compounds **19** and **21** with the 3*R* configuration, respectively (Table IV). The (*S*)-enantiomers **22** and **24** also showed higher affinity than the corresponding (*R*)-enantiomers **23** and **25**. Thus, the compounds bearing (*S*)-1-azabicyclo[2.2.2]octan-3-yl moiety were more potent than those bearing (*R*)-1-azabicyclo[2.2.2]octan-3-yl moiety. The 2*S* diastereomers **18** and **19** were slightly more potent than the corresponding 2*R* diastereomers **20** and **21**, respectively. In redard to the substituents at position 2 of the dihydrobenzofuran ring, the order of potency was dimethyl ≧ (2*S*)-methyl > (2*R*)-methyl > dihydro. Introduction of the methyl group at position 2 of the dihydrobenzofuran ring showed a considerable increase in partition coefficients (log *P*, Table IV). However, the (2*S*)-methyl compound **18** had lower lipophilicity than the (2*R*)-methyl compound **20**. These results suggest that the lipophilicity of the methyl groups at position 2 of the dihydrobenzofuran ring contributes to the pharmacological activity. Additionally, some interaction of the (2*S*)-methyl group at the same position with the 5-HT_3 receptor would contribute to the enhancement of the pharmacological activity.

Thus, it can be assumed in the case of the 5-HT_3 receptor

TABLE IV. Physical Data and 5-HT_3 Receptor Binding Affinities of the Stereoisomers **18**—**25**



Compd. No.	R^1	R^2	Isomerism Amine	Isomerism Furan	Yield (%)	$[\alpha]_D^{25}$[a] (*c* = 1.0) (Solvent)	mp (°C)	Formula	Analysis (%) Calcd (Found) C	H	N	log *P*[b] (t_R)[c]	[^{3}H]Granisetron binding K_i (nM)
18	CH_3	H	*S*	*S*	51.2	+17.0 (MeOH)	245—247 (dec.)	$C_{17}H_{21}ClN_2O_2 \cdot$ HCl	57.15 (57.31	6.21 6.29	7.84 7.86)	1.43 (12.99)	0.078
19	CH_3	H	*R*	*S*	43.0	+10.6 (MeOH)	277—281 (dec.)	$C_{17}H_{21}ClN_2O_2 \cdot$ HCl	57.15 (57.14	6.21 6.20	7.84 7.81)		0.30
20	H	CH_3	*S*	*R*	52.6	−10.6 (MeOH)	277—281 (dec.)	$C_{17}H_{21}ClN_2O_2 \cdot$ HCl	57.15 (57.08	6.21 6.17	7.84 7.80)	1.59 (14.50)	0.12
21	H	CH_3	*R*	*R*	50.6	−17.1 (MeOH)	245—247 (dec.)	$C_{17}H_{21}ClN_2O_2 \cdot$ HCl	57.15 (57.30	6.21 6.27	7.84 7.80)		0.68
22	H	H	*S*		52.7	−32.3 (MeOH)	172—174	$C_{16}H_{19}ClN_2O_2$	62.58 (62.50	6.19 6.34	9.13 9.19)	1.24 (8.54)	0.16
23	H	H	*R*		41.8	+32.0 (MeOH)	172—174	$C_{16}H_{19}ClN_2O_2$	62.58 (62.59	6.19 6.22	9.13 9.16)		1.7
24	CH_3	CH_3	*S*		59.9	+1.8 (H_2O)	292—295 (dec.)	$C_{18}H_{23}ClN_2O_2 \cdot$ HCl	58.16 (58.21	6.46 6.56	7.54 7.57)	1.69 (20.62)	0.055
25	CH_3	CH_3	*R*		60.3	−2.0 (H_2O)	292—295 (dec.)	$C_{18}H_{23}ClN_2O_2 \cdot$ HCl	58.16 (58.11	6.46 6.48	7.54 7.46)		0.29

a) The optical purity of the enantiomers, determined by HPLC, was more than 99% ee. *b*) Partition coefficient. *c*) See the experimental section.

TABLE V. 5-HT_3 Receptor Antagonistic Activities of Compounds **1**, **2**, **17**, and **24**

Compd. No.	[^{3}H]Granisetron binding K_i (nM)	BJ reflex[a] ED_{50} (μg/kg i.v.)
17	0.11	0.34 (0.27—0.47)
24	0.055	0.18 (0.13—0.23)
1[b]	0.41	0.74 (0.47—1.07)
2[b]	0.18	0.50 (0.40—0.63)

a) Serotonin was administered at a dose of 10 μg/kg i.v. 5 min posttreatment with drug at the specified dose. Values in parentheses indicate the 95% confidence limits. *b*) Reference compounds.

that there is a new lipophilic site near the (2*S*)-methyl group at position 2 of the dihydrobenzofuran ring, though the capacity of the lipophilic site is unknown. The capacity of the lipophilic site is now under investigation.

In conclusion, new potent 5-HT_3 receptor antagonists in the 2,3-dihydrobenzofuran-7-carboxyamide series were synthesized. Compound **24** showed high affinity for 5-HT_3 receptors ($K_i = 0.055$ nM) and especially potent antagonistic activity on the BJ reflex ($ED_{50} = 0.18$ μg/kg i.v.). Compound **24** was clearly more potent than the reference compounds **1** and **2** (Table V). Furthermore, in this study, we demonstrated that the methyl group with *S* absolute configuration of the *gem*-methyl substituents at position 2 of the dihydrobenzofuran ring should participate in the enhancement of the activity. The information will be of benefit in the disign of further 5-HT_3 receptor antagonists.

Experimental

Melting points were determined in open capillaries and are uncorrected. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a JEOL PS-100 spectrometer and the chemical shifts were expressed in ppm downfield from tetramethylsilane as an internal standard. Infrared (IR) spectra were recorded on a JASCO IR-810 instrument. Low-resolution mass spectra (MS) were obtained by a JMS-O1SG spectrometer. Optical rotations were obtained on a JASCO DIP-181 digital polarimeter. Elemental analysis and measurement of these spectra were performed by our laboratory. Granisetron, and zacopride were prepared by the reported methods[13a,b] in our laboratory.

General Procedure for the Preparation of 2,3-Dihydrobenzofuran-7-carboxamide and Carboxylate Derivatives Physical and spectral data of compounds **6**—**17** are listed in Tables I and II.

Method A: To a mixture of the carboxylic acid **5b**—**i**[6,7] (10 mmol), NEt_3 (10 mmol), AcOEt (30 ml) was added pivaloyl chloride (10 mmol) at −10 °C. The mixture was stirred below −5 °C for 30 min, and a solution of the appropriate amine (10 mmol) in AcOEt (5 ml) was added with stirring at −10 °C. Stirring was continued at −10 °C for 30 min, and then at room temperature for 1 h, water was added and the resulting mixture was extracted with AcOEt. The extract was washed with water, dried over $MgSO_4$ and evaporated to dryness. The residue was recrystallized and converted to the hydrochloride in the usual manner.

Method B: A mixture of the carboxylic acid **5a** (10 mmol), thionyl chloride (12 mmol), and dimethylformamide (DMF) (1 drop) in 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-azabicylo[2.2.2]octane (10 mmol) in $CHCl_3$ (or CH_3CN) (30 ml) at below 10 °C. The reaction mixture was stirred at room temperature for 1 h, and 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 **8** and **9** were prepared from the acid chloride of **5a** according to the procedure of Richardson *et al.*[14]

The diastereomeric ratios (α/β) of some compounds were confirmed to be within the range of 0.9 to 1.1 (α: shorter t_R under the reversed-phase condition, β: longer t_R under the same condition) by HPLC [apparatus, Shimadzu LC-6AD; detection, UV at 254 nm; column, ODS-M (Shimadzu Techno-Research, Inc.) 150 × 6.0 (i.d.) mm; mobile phase, 0.1 M sodium perchlorate buffer (pH 2.5)–acetonitrile (7 : 3)].

Optical Resolution of 5-Chloro-2-methyl-2,3-dihydrobenzofuran-7-carboxylic Acid (5a) A mixture of cinchonidine (10.3 g, 0.035 mol) and isopropanol (IPA) (40 ml) was added to a mixture of racemic 5-chloro-2-methy-2,3-dihydrobenzofuran-7-carboxylic acid[7] **5a** (7.4 g, 0.035 mol) and IPA (40 ml). The mixture was dissolved by warming, and was allowed to stand overnight at room temperature. The precipitates were collected by filtration, recrystallized from EtOH–MeOH (20 ml–10 ml), and then recrystallized once more from EtOH–MeOH (15 ml–8 ml) to give 1.4 g of the cinchonidine salt of (−)-**5a**, which was suspended in water (15 ml) and acidified by addition of 6 N HCl with stirring. The precipitates were collected and recrystallized from water–EtOH to afford 0.57 g of (−)-**5a**: mp 188—190 °C, $[\alpha]_D^{24} = -7.5°$ (c = 1.0, DMF) *Anal.* Calcd for $C_{10}H_9ClO_3$; C, 56.49; H, 4.27. Found: C, 56.76; H, 4.35.

A solution of cinchonine (10.3 g, 0.035 mol) in EtOH (50 ml) was added to a mixture of the racemic **5a** (7.4 g, 0.035 mol) and EtOH (50 ml). The mixture was dissolved by warming, and was allowed to stand overnight at room temperature. The precipitates were collected by filtration, recrystallized from EtOH (60 ml), and then recrystallized once more from IPA (50 ml) to give 1.3 g of the cinchonine salt of (+)-**5a**, which suspended in water (15 ml) and acidified by addition of 6 N HCl with stirring. The precipitates were collected and recrystallized from water–EtOH to afford 0.43 g of (+)-**5a**: mp 188—190 °C, $[\alpha]_D^{27} = +7.1°$ (c = 1.0, DMF). *Anal.* Calcd for $C_{10}H_9ClO_3$; C, 56.49; H, 4.27. Found: C, 56.58; H, 4.31.

General Procedure for the Preparation of Optically Active 2,3-Dihydrobenzofuran-7-carboxamide Physical and spectral data for the stereoisomers **18**—**25** are listed in Table IV. To a mixture of the carboxylic acid ((−)-**5a**, (+)-**5a**, **5h**—**i**[7]) (10 mmol), NEt_3 (10 mmol), and AcOEt (30 ml) was added pivaloyl chloride (10 mmol) at −10 °C. The mixture was stirred below 5 °C for 30 min, and a solution of an enantiomer of 3-amino-1-azabicyclo[2.2.2]octane[9] (10 mmol) in AcOEt (5 ml) was added with stirring at −10 °C. The mixture was stirred at −10 °C for 30 min, and then at room temperature for 1 h, water was added and the resulting mixture was extracted with AcOEt. The extract was washed with water, dried over $MgSO_4$ and evaporated to dryness. The residue was recrystallized and converted to the hydrochloride in the usual manner.

The enantiomeric purities of stereoisomers of compounds were confimed to be >99% ee by chiral HPLC [apparatus, Shimadzu LC-6AD; detection, UV at 254 nm; column, Chiralpac OD (Daicel Chemical Industries, Ltd.) 250 × 4.6 (i.d.) mm; mobile phase, *n*-hexane–dichloromethane–dichloroethane–IPA–diethylamine (250 : 150 : 50 : 50 : 1)].

Determination of Partition Coefficient A solution (10 ml) of a test compound (10 mg) in buffer (pH 7.4) was injected into 1-octanol (10 ml) in a shaking cell at 25 °C. The mixture was stirred at 25 °C for 4 h and centrifuged at 3000 rpm for 30 min, and the two phases were separated. The concentration of each phase was determined by HPLC [apparatus, Shimadzu LC-6AD; detection, UV at 254 nm; column, ODS-M (Shimadzu Techno-Research, Inc.) 150 × 6.0 (i.d.) mm; mobile phase, 0.1 M perchlorate buffer (pH 2.5)–acetonitrile (7 : 3); flow rate (1.2 ml/min)]. The logarithm of the partition coefficient (log *P*) was calculated as the logarithm of the ratio of the concentration of the organic phase to the cncentration of the aqueous phase. HPLC retiontion times were determined on an ODS-M column under the same conditions.

[^{3}H]Granisetron Binding [^{3}H]Granisetron binding assay was performed according to the method of Nelson and Thomas.[15] Rat cerebral cortex was homogenized in 20 volumes of 0.32 M sucrose and centrifuged at 1000 × *g* for 10 min. The supernatant was centrifuged at 40000 × *g* for 15 min. The pellet was resuspended in 20 volumes of HEPES buffer (50 mM, pH 7.4) and the suspension was incubated at 37 °C for 10 min, and the centrifuged at 40000 × *g* for 15 min. The pellet was washed and centrifuged (40000 × *g* for 15 min). The final pellet was resuspended in 30 volumes of HEPES buffer and used as tissue homogenate. The binding assay consisted of 50 μl of [^{3}H]granisetron (New England Nuclear), 50 μl of displacing drugs and 900 μl of tissue homogenate.

Following a 30 min incubation at 25 °C, the assay mixture was rapidly filtered under reduced pressure through Whatman GF/B glass filters which had been presoaked in 0.1% polyethyleneimine. Filters were washed immediately with 3×3 ml of ice-cold Tris–HCl buffer (50 mM, pH 7.4). ICS 205,930 (100 μM) was used for the determination of nonspecific binding. IC_{50} values were determined from concentration-inhibition curves. K_i values were determined from the relationship $K_i = IC_{50}/(1 + c/K_d)$, where c is the concentration of [^{3}H]granisetron and K_d is the dissociation constant of [^{3}H]granisetron.

von BJ Reflex Test The antagonism of 5-HT induced bradycardia was evaluated according to the method of Fozard.[16] Male Wistar rats weighing 350—450 g were anesthetized with urethane (1.25 g/kg i.p.). Blood pressure was recorded from the left femoral artery by means of a pressure transducer. Heart rate was monitored with a tachometer (San-ei, model 1321). The jugular vein was cannulated for intravenous injections of the test drugs and 5-HT. After completion of operative procedures, 100 units of heparin (Heparin sodium injection-N, Shimadzu) was injected intravenously. The test drug was administered 5 min before the rapid bolus injection of 5-HT, (10 or 20 μg/kg). For inhibition of 5-HT-induced changes in heart rate, statistical significance between mean values was determined with Student's t test for paired data. The criterion of statistical significance was $p < 0.05$. The ED_{50} value of the test drug was determined by a modification of the method of Waud[17] as the dose which suppressed the bradycardia by 50%.

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