Design and Synthesis of Piperazinylpyridine Derivatives as Novel 5-HT_{1A} Agonists/5-HT_3 Antagonists for the Treatment of Irritable Bowel Syndrome (IBS)

Akira Asagarasu,*[,a] Teruaki Matsui,[a] Hiroyuki Hayashi,[a] Satoru Tamaoki,[b] Yukinao Yamauchi,[b] and Michitaka Sato[a]

[a] *Synthetic Research Department, ASKA Pharmaceutical Co., Ltd.; and* [b] *Pharmacological Research Department, ASKA Pharmaceutical Co., Ltd.; 1604 Shimosakunobe, Takatsu-ku, Kawasaki 213–8522, Japan.*
Received July 31, 2008; accepted October 29, 2008; published online November 5, 2008

We have prepared a series of piperazinylpyridine derivatives for the treatment of irritable bowel syndrome (IBS). These compounds, which were designed by pharmacophore analysis, bind to both serotonin subtype 1A (5-HT_{1A}) and subtype 3 (5-HT_3) receptors. The nitrogen atom of the isoquinoline, a methoxy group and piperazine were essential to the pharmacophore for binding to these receptors. We also synthesized furo- and thienopyridine derivatives according to structure–activity relationship analyses. Compound 17c (TZB-20810) had high affinities to these receptors and exhibited 5-HT_{1A} agonistic activity and 5-HT_3 antagonistic activity concurrently, and is a promising drug for further development in the treatment of IBS.

Key words structure–activity relationship; irritable bowel syndrome; serotonin receptor

Irritable bowel syndrome (IBS) is a disease for which the main symptoms are evacuation abnormalities including diarrhea, constipation or bellyache, and IBS is not caused by intestinal organic lesion.[1,2] This disease develops as a result of mutual association of intestinal motion disorder, viscerosensory anaphylaxis and psychological and social factors.[3,4] The abdominal pain type is usually described in a patient as diarrhea-predominant, constipation-predominant or alternating stool pattern.

Serotonin receptor subtypes 3 (5-HT_3) in the intestine plays a role in intestinal contraction, secretion of intestinal juice, peristalsis and content transport, so diarrheal symptoms can be improved by administration of 5-HT_3 antagonists. Alosetron (**3**) and Cilansetron (**4**) are selective 5-HT_3 antagonists[5] useful for diarrhea-predominant IBS patients.[6]

On the other hand, because psychological and social factors are recognized to be one of the origins of IBS, the administration of benzodiazepine antianxiety agents has been investigated for IBS therapy. Buspirone (**2**), which is a serotonin receptor subtypes 1A (5-HT_{1A}) agonist,[7] is marketed for the treatment of stress-caused dyspeptic ulcers. The mechanism has been attributed to reduction in stress by the antianxiety action of buspirone acting as a 5-HT_{1A} agonist.

Since mental factors such as stress are also one of the causes of IBS, we attempted to synthesize a compound which acts as both a 5-HT_{1A} agonist and a 5-HT_3 antagonist, aiming to act on both receptors with a single compound to treat IBS. We expected that 5-HT_{1A} agonistic action would result in an antianxiety effect on the central nerve system and 5-HT_3 antagonistic action would show a peripheral alimentary canal movement control action. The combination of 5-HT_{1A} agonistic and 5-HT_3 antagonistic activities could possibly lead to original potent drugs for IBS. We tried to design and synthesis based on our pharmacophore analysis, and finally found one such compound in which both actions are shown by one compound suitable for this purpose. In this paper, the design and synthesis of such a compound acting on both 5-HT_{1A} and 5-HT_3 receptors, and its *in vitro* and *in vivo* activity are shown.

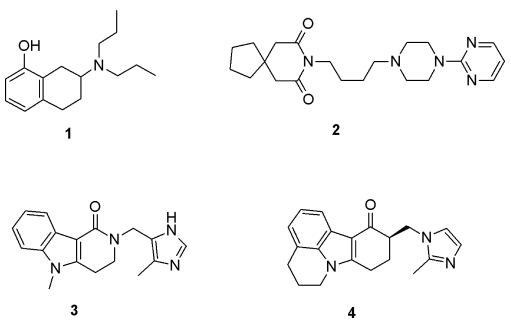


Fig. 1. 5-HT_{1A} Agonists (8-OH-DPAT (**1**), Buspirone (**2**)) and 5-HT_3 Antagonists (Alosetron (**3**), Cilansetron (**4**))

Exploration and Synthesis of the Lead Compound

First, we analyzed many structures of many 5-HT_{1A} agonists and 5-HT_3 antagonists and extracted certain pharmacophores from them. For example, from 5-HT_{1A} agonist **S-14506** (**5**)[8] we extracted a pharmacophore including an aromatic ring as the basic template, a hydrogen bond acceptor (methoxy group), a basic nitrogen that exists at a certain constant distance from the aromatic ring or the hydrogen acceptor and a bulky hydrophobic group linked by a spacer from the nitrogen. Furthermore, from 5-HT_3 antagonist Quipazine (**6**)[9,10] we extracted a pharmacophore including an aromatic ring again as the basic template similar to the 5-HT_{1A} agonist, a hydrogen bond acceptor (nitrogen) in the aromatic ring and a basic nitrogen that exists at a certain constant distance from the aromatic ring or the hydrogen acceptor.

Second, we superimposed these pharmacophores of the known 5-HT_{1A} agonist and 5-HT_3 antagonist, which had aryl piperazine as a common structure. We hypothesized that the bulky hydrophobic group linked by a spacer, as a pharmacophore of 5-HT_{1A} agonist, would not be good for binding to 5-HT_3, so this was removed from the pharmacophore model. We modelled the pharmacophore which could bind to both 5-HT_{1A} and 5-HT_3 receptors as follows: 1. An aromatic ring as the basic template, 2. A hydrogen bond acceptor (nitrogen) in the aromatic ring, 3. A basic nitrogen that exists at a certain

* To whom correspondence should be addressed. e-mail: asagarasu-a@aska-pharma.co.jp

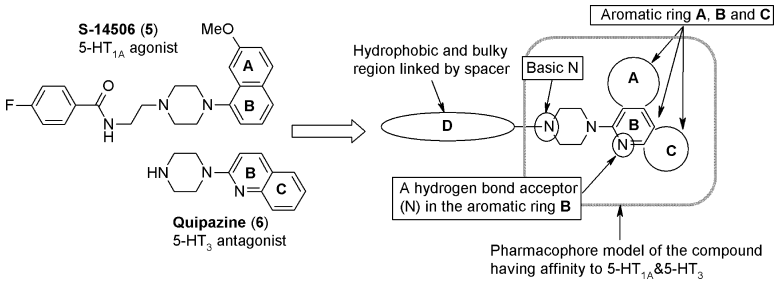


Fig. 2. Analysis of the Pharmacophore of the Compound That Binds to 5-HT_{1A} or 5-HT_3, and Setting the Pharmacophore Model of the Compound That Binds to Both Receptors 5-HT_{1A} and 5-HT_3

constant distance from the aromatic ring. In Fig. 2, regarding the aromatic ring in the pharmacophore model, rings A and B will prefer to bind to the 5-HT_{1A} receptor, whilst rings B and C will prefer to bind to the 5-HT_3 receptor. Therefore, we hypothesised that the compound which could bind to both receptors must have all rings A, B and C. Based on these analyses, we designed and synthesized compounds having both features described above which would bind to both receptors.

Synthesis of Arylpiperazine Derivatives Each arylpiperazine was synthesized by the same procedure in good yield (Chart 1). The corresponding aryl chloride, synthesized individually, was coupled with excess piperazine anhydride in ethyleneglycol at 140 °C.[9—11] In the case of *N*-alkyl type or diazabicyclo type, they were synthesized by the coupling of the arylchloride with a small excess of the amine moiety. The diazabicyclononane and diazabicyclodecane derivatives were synthesized from the corresponding L/D-Pro or piperazine-2-carboxylic acid.[12] The compounds having a benzyl unit on the nitrogen at the 1-position of piperazine were synthesized by coupling with arylpiperazine and the corresponding substituted benzylhalide. Furo- and thienopyridine derivatives were synthesized from the corresponding furan aldehyde or thiophen aldehyde followed by coupling with amine in the same way described above (Chart 2).[11]

Preliminary Studies to Find a Lead Compound Affinities of each compound binding to the 5-HT_{1A} receptor were calculated as percent inhibition (%) of binding of [^{3}H]-8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) (**1**) to the 5-HT_{1A} receptor in a CHO cell membrane sample in which human 5-HT_{1A} receptor was expressed.[13,14] Affinities of each compound binding to the 5-HT_3 receptor were calculated as percent inhibition (%) to binding of [^{3}H]BRL-43694 to the 5-HT_3 receptor in HEK-293 cell membrane sample in which human 5-HT_3 receptor was expressed.[13,14] We synthesized compounds using the pharmacophore model and performed a screening. Shown in Table 1, first, we synthesized compounds **7a** and **7b**, which fit in the pharmacophore model described above. These compounds bound strongly to the 5-HT_3 receptor, but poorly to the 5-HT_{1A} receptor. Therefore, we synthesized more compounds to analyze our preliminary understanding of the structure–activity relationship. Compound **8**, which was already synthesized by Campiani *et al.*[10] and whose derivatives had affinities to 5-HT_{1A} and 5-HT_3 receptors, fit in our model. But it showed very high affinity to 5-HT_3 receptor but medium to 5-HT_{1A}. From these results, it was assumed that the aromatic ring C (mentioned in Fig. 2) was obstructive to raise the affinity to 5-HT_{1A} receptor though was important to raise the affinity and the selectivity to 5-HT_3 receptor. Then we found compound **9a**, which is an isoquinoline derivative, to have a balanced affinity to both receptors, so chose this as a lead compound.

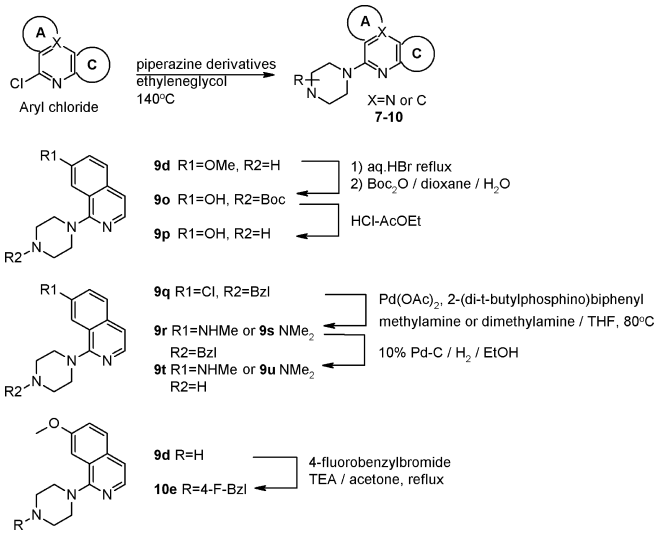
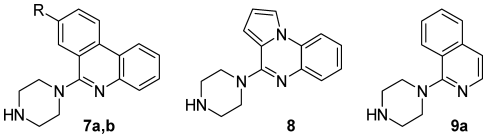


Chart 1. Synthesis of Arylpiperazine Derivatives (Aryl=Tricyclic or Isoquinolines)

Table 1. Preliminary Synthetic Study to Find Lead Compound



Entry	R	5-HT_{1A} inhibition (%)		5-HT_3 inhibition (%)	
		10^{-7} M	10^{-8} M	10^{-7} M	10^{-8} M
7a	H	41.2	−0.6	99.9	98.4
7b	OMe	6.0	N.D.[a]	99.5	92.4
8		86.4	47.6	99.9	98.9
9a		74.6	24.7	95.6	36.2

a) N.D.=no data.

Lead Optimization We modified the lead compound **9a** to optimize binding to both receptors 5-HT_{1A} and 5-HT_3. The binding inhibition activities (%) are shown in Table 2. These results showed that introduction of substituents excluding the 7-position of isoquinoline decreases the affinity to the 5-HT_{1A} receptor, and substitution on the 7-position of isoquinoline improved binding to both 5-HT_{1A} and 5-HT_3 recep-

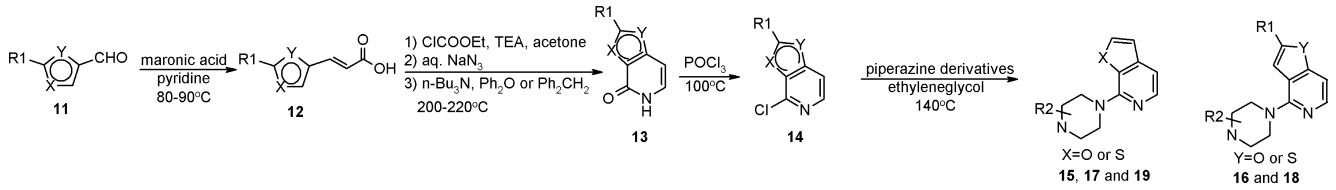
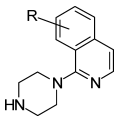


Chart 2. Synthesis of Furo- and Thienopyridine Derivatives

Table 2. The Inhibition Activity of Arylpiperazine Derivatives (1), Substitution on Isoquinoline



Entry	R	5-HT_{1A} inhibition (%)		5-HT_3 inhibition (%)	
		10^{-7} M	10^{-8} M	10^{-7} M	10^{-8} M
9a	H	74.6	24.7	95.6	36.2
9b	5-OMe	4.5	N.D.[a)]	99.1	92.2
9c	6-OMe	42.9	8.5	99.3	94.4
9d	7-OMe	91.7	49.4	97.0	47.4
9e	3-Cl	59.4	14.0	98.7	85.7
9f	5-Cl	11.8	N.D.	94.1	16
9g	6-Cl	88.9	44.7	96.5	42.9
9h	7-Cl	88.7	49.8	96.3	53.7
9i	7-F	80.4	37.1	96.8	55.4
9j	7-Br	92.8	64.7	93.4	37.7
9k	7-Me	79.7	31.1	90.1	24.1
9l	7-Ph	93.7	55.9	74.2 (at 10^{-6} M)	
9m	7-OMe, 3-Me	32.7	N.D.	80.4	N.D.
9n	7-OMe, 4-Me	23.9	N.D.	92.5	15.3
9p	7-OH	96.0	80.5	91.8	20.1
9t	7-NHMe	78.7	33.9	−0.1	−15.4
9u	7-NMe_2	93.9	53.1	18.2	N.D.

a) N.D.＝no data.

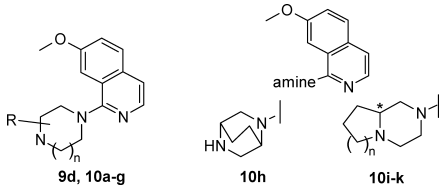
tors (compounds **9d**, **9h**). Therefore, we focused on synthesizing subsitutents at 7-position of isoquinoline. Some of these exhibited higher affinity to 5-HT_{1A} than that of compound **9a**, but had unbalanced affinities to both receptor. When R is F, Cl or Br, it has good binding affinity to both receptors, but when R is a large substituent such as Ph, it resulted in low affinity (compound **9l**). Polar substituents except for OH nearly all showed low affinities to the 5-HT_3 receptor (compounds **9t**, **9u**). Substitution on the 3- or 4-position of isoquinoline led to a low affinity to 5-HT_{1A} (compounds **9m**, **9n**).

Next, we examined the affinity to both receptors of the compounds which had subsititutions on the N of piperazine to alkyl or benzyl derivatives (Table 3). Initially, we synthesized aryl amine, using various amine units including pyrroridine derivatives, piperidine derivatives, acyclic amines and pyridines, but they had low or no affinity to both receptors (data not shown). Then we focused on substitution on piperazine. Aryl *N*-methyl piperazine (**10b**) also preserved binding affinities to both receptor, but this *N*-ethyl derivative (**10d**) resulted in a decrease in the affinities. *N*-Benzyl derivatives (**10e**, **10f**) showed low affinities especially to 5-HT_3, suggesting our pharmacophore model described above held true. As the large hydrophobic region linked from N of piperazine was a pharmacophore for 5-HT_{1A} and not for 5-HT_3, compounds **10e** and **10f** showed high 5-HT_{1A} affinity and low 5-HT_3 affinities. Changing the piperazine ring to homopiperazine (**10g**) or 2,5-diaza-bicyclo[2.2.2]octane (**10h**) resulted in a decrease in affinity. Compound **10a**, with a methyl group on the 3-position of piperazine, showed similar affinities to both receptors as compound **9d**. However, disubstituted compound **10c** showed a lower affinity than **10a**. As mentioned above that substitution on the 4-position of piperazine with methyl preserved affinities, we synthesized a compound with di-substitution on 3- and 4-position in a cyclic form. The diazabicyclo-type compound **10i** showed excellent affinity to both receptors. Interestingly, the stereo isomer **10j** also showed high affinities to both receptors but the *S* isomer (**10i**) had stronger affinities than the *R* one. Compound **10k**, with a bigger ring size, showed moderate affinity but lower than **10i** or **10j**.

Continuously to optimize the compound **9d**, we tried to change the isoquinoline moiety to another aryl group. To explore templates with a higher affinity to both receptors, we synthesized modified isoquinolines. We first synthesized compounds whose aryl moiety was phthalazine, quinazoline or naphthyridine, with N and methoxy groups at the 2-position and 7-position of isoquinoline respectively. However, these substitutions resulted in low affinities to both receptors (data was not shown). Then, we designed furo[2,3-*c*]pyridine derivatives with oxygen in the aromatic ring, corresponding to the 7-position of isoquinoline, which resulted in a relatively high affinity to both receptors (compounds **15a, 15b**, Table 4). Likewise, thieno[2,3-*c*]pyridine derivatives also showed relatively high affinities (compounds **15c, 15d**). Interestingly, the oxygen or sulfur atom of these compounds was designed to mimic the role of the methoxy group of isoquinoline derivatives, but furo[3,2-*c*]pyridine or thieno[3,2-*c*]pyridine, whose oxygen or sulfur atom was in the opposite orientation of the corresponding methoxy group of **9d**, showed relatively high affinity similarly to the furo- or thieno[2,3-*c*]pyridine derivatives though there were some differences (compound **16**[11)]). Generally, furo- or thieno[2,3-*c*]-pyridine derivatives showed slightly higher activities.

As the furo- or thienopyridine derivatives showed as high affinity as isoquinoline derivative **9d**, we built diazabicyclononane into these furo- and thienopyridine derivatives (Table 5). As expected, these compounds showed excellent high affinities to both receptors. Furo- or thieno[2,3-*c*]pyridine derivatives showed higher affinities to 5-HT_{1A} than furo- or thieno[3,2-*c*]pyridine derivatives, the same as the results of piperazine derivatives. There was little difference of the affinities between the *S*-isomer and *R*-isomer, but the *S*-isomer showed only a little higher affinity than the *R*-isomer. The diazabicyclodecane derivatives (**19**) showed similarly high affinities, but slightly lower than diazabicyclononane derivatives.

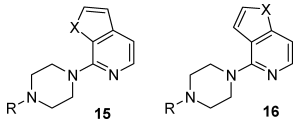
Table 3. The Inhibition Activity of Arylpiperazine Derivatives (2), Optimization of Piperazine Unit



Entry	R	*n*	5-HT$_{1A}$ inhibition (%)		5-HT$_3$ inhibition (%)	
			10^{-7} M	10^{-8} M	10^{-7} M	10^{-8} M
9d	H	1	91.7	49.4	97.0	47.4
10a	3-Me	1	94.8	66.5	93.1	20.0
10b	4-Me	1	91.7	51.5	94.4	14.6
10c	3,5-Me$_2$	1	87.8	47.9	27.6 (at 10^{-6} M)	
10d	4-Et	1	86.7	32.4	19.7	−2.3
10e	4-F	1	81.9	26.7	−2.0	N.D.[a]
10f	4-methylenedioxy	1	94.8	61.4	42.2	5.8
10g	H	2	62.5	16.1	96.3	43.3
10h			4.0	N.D.	68.6	N.D.
10i (*S*-isomer)		1	96.7	80.2	99.8	91.8
10j (*R*-isomer)		1	98.9	83.3	97.9	57.9
10k (racemate)		2	95.8	68.0	74.4	18.1

a) N.D.=no data.

Table 4. The Inhibition Activity of Furo- and Thienopyridine Derivatives



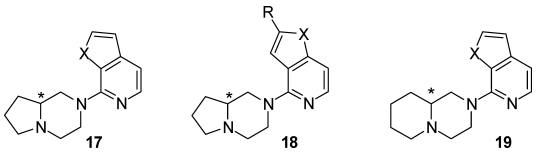
Entry	R	X	5-HT$_{1A}$ inhibition (%)		5-HT$_3$ inhibition (%)	
			10^{-7} M	10^{-8} M	10^{-7} M	10^{-8} M
15a	H	O	86.7	41.5	80.8	7.5
15b	Me	O	83.7	41.6	86.4	11.2
15c	H	S	89.1	49.4	96.7	45.8
15d	Me	S	90.1	52.2	97.5	53.9
16a	H	O	69.9	26.3	50.3	7.6
16b	Me	O	72.4	23.6	65.6	3.1
16c	H	S	74.8	24.6	90.7	11.9
16d	Me	S	78.3	35.8	93.3	22.2

For a second screening, *in vitro* functional investigations were carried out for these compounds with high affinities to both receptors. A [^{35}S]GTPγS binding assay and a contraction inhibition on ileum of guinea-pig were performed (Table 6).[13] In this [^{35}S]GTPγS binding assay, every compound of this series had about 90% of binding activity, and were confirmed to be full 5-HT$_{1A}$ agonists. For contraction inhibition, almost every compound showed 90—100% inhibition at 1 μmol/l, confirming that they were 5-HT$_3$ antagonists. In particular, **17a** and **17c** showed excellent results for these *in vitro* functional assays.

For a third screening, two *in vivo* functional examinations were carried out in rat: measurement of 5-HT$_{1A}$ agonistic activity (lower lip retraction: LLR, flat body posture: FBP and change in rectal temperature: ΔT), and inhibition of the Bezold–Jarisch (BJ) reflection caused by 5-HT (Table 7).[13,14] Compound **17a** showed excellent results comparing LLR, FBP and ΔT. So **17a** was confirmed as a 5-HT$_{1A}$ agonist *in vivo*. In contrast, **17c** showed a low FBP score, and **18c** showed a low score for all measurements. In inhibition of the BJ reflection test, these compounds showed about 50% inhibitions from a low dosage, and then they were confirmed as a 5-HT$_3$ antagonist *in vivo*. As mentioned above, we confirmed that this series of furo- and thienopyridines with diazabicyclononane were 5-HT$_{1A}$ agonists/5-HT$_3$ antagonists *in vitro*/*vivo*.

Among them, **17a**(=**TZB-20810**) showed strong 5-HT$_{1A}$ agonostic/5-HT$_3$ antagonistic action. Therefore, we studied the IC$_{50}$ value of **17a** for other receptors (Table 8). In rat α_1 human D$_2$ and guinea-pig 5-HT$_4$, its IC$_{50}$ value were very low (>100 nmol/l).[15] In rat 5-HT$_2$ and α_2 receptors, its IC$_{50}$ value were 41 nM. We thought that the effect of 5-HT$_2$ and α_2

Table 5. The Inhibition Activity of Furo- and Thienopyridine with Diazabicyclo Derivatives



Entry	X	Stereo[a]	R	5-HT_{1A} inhibition (%)		5-HT_3 inhibition (%)	
				10^{-7} M	10^{-8} M	10^{-7} M	10^{-8} M
17a	O	*S*		95.4	63.4	98.9	83.7
17b	O	*R*		78.0	33.7	91.2	33.4
17c	S	*S*		91.4	45.8	99.5	97.0
17d	S	*R*		90.6	49.8	100.1	81.9
18a	O	*S*	H	72.7	37.3	96.4	60.4
18b	O	*R*	H	56.4	10.9	82.4	23.5
18c	S	*S*	H	85.5	69.2	99.3	82.9
18d	S	*R*	H	75.4	23.4	92.5	26.6
18e	S	*S*	Me	79.7	33.9	95.9	51.2
19a	O	*rac.*		76.0	27.3	84.9	15.8
19b	S	*rac.*		81.0	32.3	99.4	58.4

a) *rac.*=racemate.

Table 6. 5-HT_{1A} Agonistic Action and 5-HT_3 Antagonistic Action *in Vitro*

Entry	5-HT_{1A} agonist		5-HT_3 antagonist	
	[^{35}S]GTPγS		Ileum (*in vitro*) inhibit. (%)	
	EC_{50}(nM)	E_{max}(%)	1 μM	0.1 μM
9d	47.5	84.7	67.7	8.2
15c	68.9	92.2	85.1	−16.0
17a	27.5	90.8	89.2	81.5
17c	56.3	97.0	86.7	86.7
17d	92.4	79.9	87.8	50.3
18c	70.4	82.1	100.0	77.0
18e	12.8	93.3	95.2	10.5

Table 7. 5-HT_{1A} Agonistic Action and 5-HT_3 Antagonistic Action *in Vivo*

Entry	5-HT_{1A}[a]			5-HT_3	
	Max score		ΔT[d] (−°C)	BJ reflection (i.v.)	
	LLR[b]	FBP[c]		mg/kg	% inhibit.
17a	3.0	3.0	2.1	0.03	64.9
				0.01	46.1
17c	3.0	0.4	1.9	0.003	61.5
				0.001	21.5
18c	1.6	0.2	1.1	0.003	57.6
				0.001	34.1

a) rat 10 mg/kg, s.c.; *b*) LLR=lower lip retraction; *c*) FBP=flat body posture; *d*) ΔT=change in rectal temperature.

would not be appeared, because assumed IC_{50} value of **17a** of 5-HT_{1A} receptor and 5-HT_3 receptor were less than 10 nM, and the dosage of efficacy would be able to estimate lower by owning both actions together. But higher selectivity would be hoped for, we keep researching continuously.

We then performed a model experiment in mouse of diarrhea caused by serotonin. Compound **17a**(=**TZB-20810**) showed a dose dependent improvement of score (data not shown). In these functional examinations *in vivo*, it was confirmed that compound **17a** had both 5-HT_{1A} agonistic action and 5-HT_3 antagonistic action.

Table 8. *In Vitro* Binding Assays (IC_{50}[a]) to Other Receptors

Entry	Rat α_1	Rat α_2	Human D_2	Rat 5-HT_2	gp[b] 5-HT_4
17a (=TZB-20810)	>1	0.041	>1	0.041	>1

a) IC_{50}; μM; *b*) gp=guinea pig.

Conclusion

Our initial aim was to search for compounds with both gastrointestinal motor inhibition and antianxiety effects for the treatment of diarrhea-type IBS, having both 5-HT_3 antagonistic action and 5-HT_{1A} agonistic action. By superposition of the pharmacophores of 5-HT_{1A} agonists and 5-HT_3 antagonists with arylpiperazine moiety as a common structure, we found compound **9a**, an isoquinoline derivative, had affinity to both receptors. We further optimized this isoquinoline derivative to discover furo- and thienopyridine derivatives with diazabicyclononanes. These showed high affinity to both receptors. Compound **17a**(=**TZB-20810**) showed 5-HT_{1A} agonistic/5-HT_3 antagonistic actions concurrently in the *in vitro*/*vivo* functional assay.

Because the compound had 5-HT_{1A} agonistic action and 5-HT_3 antagonistic action concurrently, the maximum medicinal effect is higher than Alosetron, 5-HT_3 antagonist.[13] Even if it was weak as the action for 5-HT_3 antagonist, the medicinal effect could be shown. Therefore, we thought that the ischemic colitis that caused by over-dose of 5-HT_3 antagonist could be evaded. Thus compound **17a**(=**TZB-20810**) was selected for advanced evaluation for the treatment of IBS.

Experimental

Unless otherwise noted, all nonaqueous reactions were carried out under an Ar atmosphere using commercial grade solvents and reagent. ^{1}H-NMR spectra were recorded on a JEOL JNM-ECP 400. Chemical shifts are reported in ppm relative to tetramethylsilane, using the following abbrevia-

tions: s, singlet; d, doublet; t, triplet; dd, doublet of doublet, *etc.*; m, multiplet; br; broad. Coupling constants (*J*) are reported in hertz (Hz) where relevant. Mass spectrometric analyses were obtained on Shimazu GC/MS QP-5000, with electrospray ionization methodology.

Synthesis of 7-Chlorofuro[2,3-*c*]pyridine (14). General Procedure for Chlorofuropyridines and Chlorothienopyridines. Step 1 A solution of 3-furaldehyde (10.0 g, 104 mmol) and malonic acid (15.0 g, 144 mmol) in pyridine (12 ml) was heated under stirring at 80—90 °C for 2 h. The solution was poured in ice water, acidified with 1 N hydrochloric acid. The precipitates were collected by filtration, dissolved in ethyl acetate and washed with 1 N hydrochloric acid. The organic layer was dried over anhydrous magnesium sulfate and concentrated. The residue was recrystallized from ethyl acetate–*n*-hexane to provide 11.78 g (82%) of 3-furan-3-ylacrylic acid (**12**). ^{1}H-NMR ($CDCl_3$) δ: 7.70—7.67 (2H, m), 7.45 (1H, s), 6.62—6.61 (1H, m), 6.16 (1H, d, J=15.8 Hz). MS *m/z*: 138 (M^+, base).

Step 2 To a solution of 3-furan-3-ylacrylic acid (**12**, 5.0 g, 36 mmol) and triethylamine (4.3 g, 43 mmol) in acetone (50 ml), ethyl chlorocarbonate (5.2 g, 48 mmol) was dropped slowly under cooling with ice, and the mixture was stirred for 30 min under cooling with ice. Then an aqueous solution (15 ml) of sodium azide (3.5 g, 69 mmol) was dropped, followed by stirring for 1 h under cooling with ice. After addition of 150 ml of ice water, the solution was extracted with benzene, dried over anhydrous magnesium sulfate and concentrated to about 20 ml under reduced pressure, while keeping the liquid temperature no higher than 30 °C. This solution was dropped into diphenylmethane (40 ml) and tributylamine (7 ml) which had been heated to 220 °C over 1.5 h while distilling the benzene off to maintain the temperature of 220 °C. After cooling of the mixture, *n*-hexane was added. The precipitates were collected by filtration, washed with ethyl acetate and dried to provide 3.2 g (64%) of 6*H*-furo[2,3-*c*]pyridin-7-one (**13**) as pale-yellow solid. ^{1}H-NMR (DMSO-d_6) δ: 11.50 (1H, br s), 8.07 (1H, d, J=1.9 Hz), 6.86 (1H, d, J=1.9 Hz), 6.50 (1H, d, J=6.9 Hz). MS *m/z*: 135 (M^+).

Step 3 To phosphorus oxychloride (16.0 g, 104 mmol), 6*H*-furo[2,3-*c*]pyridin-7-one (**13**, 3.10 g, 22.9 mmol) was added and the mixture was heated under reflux for 1.5 h. The solution was poured on ice, neutralized with saturated aqueous sodium hydrogen carbonate solution and extracted with chloroform. The extract was dried over anhydrous magnesium sulfate, and the solvent was distilled off under reduced pressure. The residue was purified on silica gel column chromatography (*n*-hexane : ethyl acetate=1 : 1) to provide 2.36 g (67%) of 7-chlorofuro[2,3-*c*]pyridine (**14**) as pale-yellow crystal. ^{1}H-NMR ($CDCl_3$) δ: 8.19 (1H, d, J=5.4 Hz), 7.81 (1H, d, J=1.9 Hz), 7.49 (1H, d, J=5.4 Hz), 6.87 (1H, d, J=2.3 Hz). MS *m/z*: 153 (M^+).

Synthesis of 7-Piperazin-1-ylfuro [2,3-*c*]pyridine (15a). General Procedure for Arylpiperazines To a solution of anhydrous piperazine (8.60 g, 100 mmol) in ethylene glycol (100 ml), 7-chlorofuro[2,3-*c*]pyridine (**14**, 1.53 g, 10.0 mmol) was added, and the mixture was stirred at 140 °C overnight. After cooling, the mixture was washed with saturated aqueous sodium hydrogencarbonate solution, and extracted with chloroform. The organic layer was dried over anhydrous magnesium sulfate, and the solvent was distilled off under reduced pressure. The residue was purified on silica gel column chromatography (methanol : chloroform=1 : 3) to provide 1.52 g (75%) of the title compound (**15a**) as oil. ^{1}H-NMR ($CDCl_3$) δ: 7.96 (1H, d, J=5.5 Hz), 7.63 (1H, d, J=2.3 Hz), 6.97 (1H, d, J=5.4 Hz), 6.72 (1H, d, J=1.9 Hz), 3.83—3.80 (4H, m), 3.06 (4H, t, J=5.0 Hz). MS *m/z*: 203 (M^+), 135.

1-Piperazin-1-ylisoquinoline (9a) This compound was synthesized using the same procedure as for **15a** starting with 1-chloroisoquinoline and anhydrous piperazine. ^{1}H-NMR ($CDCl_3$) δ: 8.15 (1H, d, J=5.9 Hz), 8.11 (1H, d, J=8.4 Hz), 7.75 (1H, d, J=8.1 Hz), 7.61 (1H, ddd, J=1.1, 7.0, 8.1 Hz), 7.51 (1H, ddd, J=1.1, 7.0, 8.4 Hz), 7.25 (1H, d, J=5.9 Hz), 3.41—3.34 (4H, m), 3.18—3.13 (4H, m). MS *m/z*: 213 (M^+), 145.

5-Methoxy-1-piperazin-1-ylisoquinoline (9b) This compound was synthesized using the same procedure as for **15a** starting with 1-chloro-5-methoxyisoquinoline and anhydrous piperazine. ^{1}H-NMR ($CDCl_3$) δ: 8.16 (1H, d, J=6.2 Hz), 7.67 (1H, d, J=8.5 Hz), 7.63 (1H, dd, J=0.8, 5.8 Hz), 7.41 (1H, t, J=7.7 Hz), 6.94 (1H, d, J=7.7 Hz), 3.99 (3H, s), 3.39—3.31 (4H, m), 3.18—3.11 (4H, m). MS *m/z*: 243 (M^+), 174.

6-Methoxy-1-piperazin-1-ylisoquinoline (9c) This compound was synthesized using the same procedure as for **15a** starting with 1-chloro-6-methoxyisoquinoline and anhydrous piperazine. ^{1}H-NMR ($CDCl_3$) δ: 8.10 (1H, d, J=5.9 Hz), 8.01 (1H, d, J=9.2 Hz), 7.16 (1H, d, J=5.9 Hz), 7.13 (1H, dd, J=2.6, 9.2 Hz), 7.02 (1H, d, J=2.6 Hz), 3.93 (3H, s), 3.38—3.32 (4H, m), 3.18—3.12 (4H, m). MS *m/z*: 243 (M^+), 187.

7-Methoxy-1-piperazin-1-ylisoquinoline (9d) This compound was synthesized using the same procedure as for **15a** starting with 1-chloro-7-methoxyisoquinoline and anhydrous piperazine. ^{1}H-NMR ($CDCl_3$) δ: 8.08 (1H, d, J=5.5 Hz), 7.68 (1H, d, J=9.2 Hz), 7.42 (1H, d, J=2.6 Hz), 7.29 (1H, dd, J=2.6, 9.2 Hz), 7.23 (1H, d, J=5.5 Hz), 3.94 (3H, s), 3.37—3.30 (4H, m), 3.20—3.13 (4H, m). MS *m/z*: 243 (M^+), 174.

3-Chloro-1-piperazin-1-ylisoquinoline Dihydrochloride (9e) This compound was synthesized using the same procedure as for **15a** starting with 1,3-dichloroisoquinoline and anhydrous piperazine. ^{1}H-NMR (DMSO-d_6) δ: 8.08 (1H, d, J=8.5 Hz), 7.90 (1H, d, J=8.1 Hz), 7.78—7.75 (1H, m), 7.64—7.62 (1H, m), 7.60 (1H, s), 3.60—3.57 (4H, m), 3.29 (4H, br s). MS *m/z*: 247 (M^+), 179.

5-Chloro-1-piperazin-1-ylisoquinoline (9f) This compound was synthesized using the same procedure as for **15a** starting with 1,5-dichloroisoquinoline and anhydrous piperazine. ^{1}H-NMR ($CDCl_3$) δ: 8.25 (1H, d, J=5.8 Hz), 8.04 (1H, td, J=1.2, 8.5 Hz), 7.69 (1H, dd, J=1.2, 7.3 Hz), 7.64 (1H, dd, J=1.2, 5.8 Hz), 7.42 (1H, dd, J=7.3, 8.5 Hz), 3.41—3.35 (4H, m), 3.18—3.13 (4H, m). MS *m/z*: 247 (M^+), 179.

6-Chloro-1-piperazin-1-ylisoquinoline (9g) This compound was synthesized using the same procedure as for **15a** starting with 1,6-dichloroisoquinoline and anhydrous piperazine. ^{1}H-NMR ($CDCl_3$) δ: 8.16 (1H, d, J=5.8 Hz), 8.03 (1H, d, J=8.9 Hz), 7.73 (1H, d, J=1.9 Hz), 7.44 (1H, dd, J=1.9, 8.9 Hz), 7.16 (1H, d, J=5.8 Hz), 3.40—3.34 (4H, m), 3.17—3.12 (4H, m). MS *m/z*: 247 (M^+), 179.

7-Chloro-1-piperazin-1-ylisoquinoline (9h) This compound was synthesized using the same procedure as for **15a** starting with 1,7-dichloroisoquinoline and anhydrous piperazine. ^{1}H-NMR ($CDCl_3$) δ: 8.16 (1H, d, J=5.8 Hz), 8.07 (1H, d, J=1.9 Hz), 7.70 (1H, d, J=8.5 Hz), 7.55 (1H, dd, J=1.9, 8.5 Hz), 7.23 (1H, d, J=5.8 Hz), 3.41—3.33 (4H, m), 3.20—3.14 (4H, m). MS *m/z*: 247 (M^+), 179.

7-Fluoro-1-piperazin-1-ylisoquinoline (9i) This compound was synthesized using the same procedure as for **15a** starting with 1-chloro-7-fluoroisoquinoline and anhydrous piperazine. ^{1}H-NMR ($CDCl_3$) δ: 8.14 (1H, d, J=5.8 Hz), 7.79—7.69 (2H, m), 7.43—7.37 (1H, m), 7.27—7.24 (1H, m), 3.38—3.32 (4H, m), 3.19—3.14 (4H, m). MS *m/z*: 231 (M^+), 163.

7-Bromo-1-piperazin-1-ylisoquinoline (9j) This compound was synthesized using the same procedure as for **15a** starting with 7-bromo-1-chloroisoquinoline and anhydrous piperazine. ^{1}H-NMR ($CDCl_3$) δ: 8.24—8.22 (1H, m), 8.16 (1H, d, J=5.8 Hz), 7.67 (1H, dd, J=1.9, 8.5 Hz), 7.61 (1H, d, J=8.9 Hz), 7.26—7.19 (1H, m), 3.36—3.32 (4H, m), 3.16—3.12 (4H, m). MS *m/z*: 292 (M^+), 235, 223.

7-Methyl-1-piperazin-1-ylisoquinoline (9k) This compound was synthesized using the same procedure as for **15a** starting with 1-chloro-7-methylisoquinoline and anhydrous piperazine. ^{1}H-NMR ($CDCl_3$) δ: 8.10 (1H, d, J=5.8 Hz), 7.88 (1H, s), 7.66 (1H, d, J=8.1 Hz), 7.45 (1H, dd, J=1.5, 8.1 Hz), 7.22 (1H, d, J=5.8 Hz), 3.39—3.32 (4H, m), 3.20—3.13 (4H, m). MS *m/z*: 227 (M^+), 159.

7-Phenyl-1-piperazin-1-ylisoquinoline (9l) This compound was synthesized using the same procedure as for **15a** starting with 1-chloro-7-phenylisoquinoline and anhydrous piperazine. ^{1}H-NMR ($CDCl_3$) δ: 8.30 (1H, s), 8.16 (1H, d, J=5.8 Hz), 7.90—7.80 (2H, m), 7.72—7.67 (2H, m), 7.54—7.48 (2H, m), 7.44—7.39 (1H, m), 7.28 (1H, d, J=5.8 Hz), 3.47—3.38 (4H, m), 3.21—3.14 (4H, m). MS *m/z*: 289 (M^+), 220.

7-Methoxy-3-methyl-1-piperazin-1-ylisoquinoline (9m) This compound was synthesized using the same procedure as for **15a** starting with 1-chloro-7-methoxy-3-methylisoquinoline and anhydrous piperazine. ^{1}H-NMR ($CDCl_3$) δ: 7.58 (1H, d, J=8.9 Hz), 7.36 (1H, d, J=2.3 Hz), 7.23 (1H, dd, J=2.3, 8.9 Hz), 7.05 (1H, s), 3.92 (3H, s), 3.36—3.32 (4H, m), 3.18—3.14 (4H, m), 2.54 (3H, s). MS *m/z*: 257 (M^+), 188.

7-Methoxy-4-methyl-1-piperazin-1-ylisoquinoline (9n) This compound was synthesized using the same procedure as for **15a** starting with 1-chloro-7-methoxy-4-methylisoquinoline and anhydrous piperazine. ^{1}H-NMR ($CDCl_3$) δ: 7.92 (1H, d, J=0.7 Hz), 7.81 (1H, d, J=9.2 Hz), 7.49 (1H, d, J=2.7 Hz), 7.40 (1H, dd, J=2.7, 9.2 Hz), 3.95 (3H, s), 3.31—3.26 (4H, m), 3.18—3.14 (4H, m), 2.49 (3H, d, J=0.7 Hz). MS *m/z*: 257 (M^+), 201, 188.

7-Hydroxy-1-piperazin-1-ylisoquinoline Hydrochloride (9p). Step 1 A mixture of **9d** (4.00 g, 16.4 mmol) and 47% aqueous hydrobromic acid (40 ml) was heated under reflux for 2 h. After cooling, the residue was made alkaline with 5 N aqueous sodium hydroxide solution, followed by addition of 1,4-dioxane (30 ml) and di-*tert*-butyldicarbonate (3.94 g, 18.1 mmol) and 1 h stirring at room temperature. The solution was extracted with ethyl acetate, washed with saturated brine, dried over anhydrous magnesium sulfate and the solvent was distilled off under reduced pressure. The residue was purified on silica gel column chromatography (methanol : chloroform=1 : 19) to provide 4.63 g (85%) of 1-(4-*tert*-butoxycarbonylpiperazin-1-yl)-7-

hydroxyisoquinoline (**9o**): ^{1}H-NMR ($CDCl_3$) δ: 8.03 (1H, d, J=5.5 Hz), 7.70 (1H, d, J=8.8 Hz), 7.43 (1H, d, J=2.6 Hz), 7.30—7.23 (2H, m), 3.72—3.64 (4H, m), 3.34—3.26 (4H, m), 1.51 (9H, s). MS m/z: 329 (M^+), 173.

Step 2 A mixture of 1-(4-*tert*-butoxycarbonylpiperazin-1-yl)-7-hydroxyisoquinoline (**9o**, 70 mg, 0.21 mmol) and 4 N hydrochloric acid-ethyl acetate solution (3 ml) was stirred for 1 h at room temperature. Precipitates were collected by filtration and washed with ethyl acetate to provide 43 mg (78%) of title compound. ^{1}H-NMR (CD_3OD) δ: 8.02 (1H, d, J=8.5 Hz), 7.78 (1H, d, J=6.6 Hz), 7.72 (1H, d, J=6.6 Hz), 7.64—7.56 (2H, m), 4.06—3.98 (4H, m), 3.68—3.59 (4H, m). MS m/z: 229 (M^+), 173.

7-Dimethylamino-1-piperazin-1-ylisoquinoline (9u). General Procedure of 7-Methylamino and 7-Dimethyaminoisoquinoline Derivatives. Step 1 A mixture of 1,7-dichloroisoquinoline (3.96 g, 20.0 mmol) and benzylpiperazine (10.5 g, 59.6 mmol) was stirred at 150 °C for 3 h. Water was added to the residue, followed by extraction with chloroform, washing with saturated aqueous sodium hydrogencarbonate solution and drying over anhydrous magnesium sulfate. Distilling the solvent off under reduced pressure, the residue was purified on silica gel column chromatography (ethyl acetate) to provide 4.78 g (71%) of 1-(4-benzylpiperazin-1-yl)-7-chloroisoquinoline (**9q**): ^{1}H-NMR ($CDCl_3$) δ: 8.14 (1H, d, J=5.4 Hz), 8.04 (1H, d, J=1.9 Hz), 7.68 (1H, d, J=8.5 Hz), 7.53 (1H, dd, J=2.3, 8.8 Hz), 7.39—7.23 (5H, m), 7.20 (1H, d, J=5.8 Hz), 3.64 (2H, s), 3.41 (4H, t, J=5.6 Hz), 2.73 (4H, t, J=4.8 Hz). MS m/z: 337 (M^+), 191, 159, 91.

Step 2 To a solution of 1-(4-benzylpiperazin-1-yl)-7-chloroisoquinoline (**9s**, 1.0 g, 3.0 mmol) in tetrahydrofuran (30 ml), palladium acetate (20 mg), 2-(di-*tert*-butylphosphino)biphenyl (48 mg, 0.16 mmol), sodium-*tert*-butoxide (0.45 g, 4.7 mmol) and 2.0 M dimethylamine-tetrahydrofuran solution (5 ml) were added and sealed in a tube. This mixture was stirred at 80 °C for 16 h. Removing the insoluble matter by filtration, the solvent was distilled off under reduced pressure and the residue was purified on silica gel column chromatography (methanol : chloroform=1 : 19) to provide 261 mg (25%) of 1-(4-benzylpiperazin-1-yl)-7-dimethylaminoisoquinoline (**9s**). ^{1}H-NMR ($CDCl_3$) δ: 7.94 (1H, d, J=5.7 Hz), 7.63 (1H, d, J=9.0 Hz), 7.45—7.20 (6H, m), 7.18 (1H, d, J=5.4 Hz), 7.12 (1H, d, J=2.3 Hz), 3.69 (2H, s), 3.49 (4H, t, J=4.8 Hz), 3.06 (6H, s), 2.77 (4H, t, J=5.0 Hz). MS m/z: 346 (M^+), 330, 200, 187, 91.

Step 3 To a solution of 1-(4-benzylpiperazin-1-yl)-7-dimethylaminoisoquinoline (**9s**, 0.25 g, 0.72 mmol) in ethanol (20 ml), 10% palladium-on carbon (50 mg) was added. This mixture was stirred for 16 h at room temperature under hydrogen atmosphere. After filtration, the filtrate was distilled off under reduced pressure. The residue was purified on silica gel column chromatography (methanol : chloroform=1 : 9) to provide 46 mg (25%) of title compound (**9u**). ^{1}H-NMR ($CDCl_3$) δ: 7.95 (1H, d, J=5.4 Hz), 7.64 (1H, d, J=8.9 Hz), 7.27 (1H, dd, J=2.3, 8.9 Hz), 7.16 (1H, d, J=5.4 Hz), 7.12 (1H, d, J=2.3 Hz), 3.39 (4H, t, J=5.0 Hz), 3.19 (4H, t, J=5.0 Hz), 3.07 (6H, s). MS m/z: 256 (M^+), 200, 187.

7-Methylamino-1-piperazin-1-ylisoquinoline (9t) This compound was synthesized using the same procedure as for **9u** using 1-(4-benzylpiperazin-1-yl)-7-chloroisoquinoline and methylamine. ^{1}H-NMR ($CDCl_3$) δ: 7.93 (1H, d, J=5.8 Hz), 7.57 (1H, d, J=8.9 Hz), 7.17 (1H, d, J=5.4 Hz), 7.01 (1H, dd, J=1.3, 8.9 Hz), 6.90 (1H, d, J=1.9 Hz), 3.56 (4H, t, J=5.0 Hz), 3.33 (4H, t, J=5.0 Hz), 2.94 (3H, s). MS m/z: 242 (M^+), 198, 186, 173.

7-Methoxy-1-(3-methylpiperazin-1-yl)isoquinoline (10a) This compound was synthesized using the same procedure as for **15a** starting with 1-chloro-7-methoxyisoquinoline and 2-methylpiperazine. ^{1}H-NMR ($CDCl_3$) δ: 8.07 (1H, d, J=5.8 Hz), 7.68 (1H, d, J=8.9 Hz), 7.40 (1H, d, J=2.7 Hz), 7.29 (1H, dd, J=2.7, 8.9 Hz), 7.23 (1H, d, J=5.8 Hz), 3.94 (3H, s), 3.70—3.62 (2H, m), 3.30—3.17 (3H, m), 3.08—2.99 (1H, m), 2.80—2.71 (1H, m), 1.20 (3H, d, J=6.6 Hz). MS m/z: 257 (M^+), 175.

7-Methoxy-1-(4-methylpiperazin-1-yl)isoquinoline (10b) This compound was synthesized using the same procedure as for **15a** starting with 1-chloro-7-methoxyisoquinoline and 1-methylpiperazine. ^{1}H-NMR ($CDCl_3$) δ: 8.07 (1H, d, J=5.8 Hz), 7.68 (1H, d, J=8.9 Hz), 7.39 (1H, d, J=2.7 Hz), 7.29 (1H, dd, J=2.7, 8.9 Hz), 7.22 (1H, d, J=5.8 Hz), 3.94 (3H, s), 3.48—3.40 (4H, m), 2.77—2.69 (4H, m). MS m/z: 257 (M^+), 187.

1-(3,5-Dimethylpiperazin-1-yl)-7-methoxyisoquinoline (10c) This compound was synthesized using the same procedure as for **15a** starting with 1-chloro-7-methoxyisoquinoline and 2,6-dimethylpiperazine. ^{1}H-NMR ($CDCl_3$) δ: 8.07 (1H, d, J=5.8 Hz), 7.68 (1H, d, J=8.9 Hz), 7.40 (1H, d, J=2.7 Hz), 7.29 (1H, dd, J=2.7, 8.9 Hz), 7.21 (1H, d, J=5.8 Hz), 3.93 (3H, s), 3.70—3.62 (2H, m), 3.33—3.23 (2H, m), 2.66—2.57 (2H, m), 1.17 (6H, d, J=6.6 Hz). MS m/z: 271 (M^+), 187.

1-(4-Ethylpiperazin-1-yl)-7-methoxyisoquinoline (10d) This compound was synthesized using the same procedure as for **15a** starting with 1-chloro-7-methoxyisoquinoline and 1-ethylpiperazine. ^{1}H-NMR ($CDCl_3$) δ: 8.07 (1H, d, J=5.8 Hz), 7.68 (1H, d, J=8.7 Hz), 7.40 (1H, d, J=2.5 Hz), 7.31—7.25 (1H, m), 7.21 (1H, d, J=5.8 Hz), 3.48—3.39 (4H, m), 2.79—2.69 (4H, m), 2.55 (2H, q, J=7.2 Hz), 1.17 (3H, t, J=7.2 Hz). MS m/z: 271 (M^+), 187.

1-[4-(4-Fluorobenzyl)piperazin-1-yl]-7-methoxyisoquinoline (10e) To a solution of **9d** (50 mg, 0.21 mmol) in acetone (10 ml), 4-fluorobenzyl bromide (0.10 g, 0.53 mmol) and triethylamine (0.10 g, 0.99 mmol) were added and heated under reflux for 5 h. Distilling the solvent off under reduced pressure, the residue was purified on silica gel column chromatography (methanol : chloroform=1 : 19) to provide 52 mg (79%) of title compound. ^{1}H-NMR ($CDCl_3$) δ: 8.07 (1H, d, J=5.5 Hz), 7.68 (1H, d, J=9.2 Hz), 7.41—7.32 (3H, m), 7.30—7.26 (1H, m), 7.21 (1H, d, J=5.5 Hz), 7.06—6.99 (2H, m), 3.93 (3H, s), 3.61 (2H, s), 3.45—3.35 (4H, m), 2.77—2.67 (4H, m). MS m/z: 351 (M^+), 187.

1-(4-Benzo[1,3]dioxol-5-ylmethylpiperazin-1-yl)-7-methoxyisoquinoline (10f) This compound was synthesized using the same procedure as for **15a** starting with 1-chloro-7-methoxyisoquinoline and 1-benzo[1,3]dioxol-5-ylmethyl-piperazine. ^{1}H-NMR ($CDCl_3$) δ: 8.07 (1H, d, J=5.8 Hz), 7.97 (1H, d, J=9.3 Hz), 7.13 (1H, d, J=5.8 Hz), 7.09 (1H, dd, J=2.7, 9.3 Hz), 7.00 (1H, d, J=2.7 Hz), 6.91 (1H, s), 6.77 (2H, t, J=7.2 Hz), 5.94 (2H, s), 3.91 (3H, s), 3.53 (2H, s), 3.39 (4H, m), 2.69 (4H, t, J=4.5 Hz). MS m/z: 377 (M^+), 187, 174, 135.

1-[1,4]Diazepan-1-yl-7-methoxyisoquinoline (10g) This compound was synthesized using the same procedure as for **15a** starting with 1-chloro-7-methoxyisoquinoline and homopiperazine. ^{1}H-NMR ($CDCl_3$) δ: 8.02—7.99 (2H, m), 7.11—7.09 (2H, m), 7.00 (1H, d, J=2.3 Hz), 3.92 (3H, s), 3.76 (2H, t, J=5.4 Hz), 3.72 (2H, t, J=5.8 Hz), 3.27 (2H, t, J=5.4 Hz), 3.16 (2H, t, J=5.7 Hz), 2.75 (1H, br s), 2.00—1.95 (2H, m). MS m/z: 257 (M^+), 242, 201, 187.

1-(2,5-Diazabicyclo[2.2.2]octan-1-yl)-7-methoxyisoquinoline (10h) This compound was synthesized using the same procedure as for **15a** starting with 1-chloro-7-methoxyisoquinoline and 2,5-diazabicyclo[2.2.2]octane. ^{1}H-NMR (DMSO-d_6) δ: 7.97 (1H, d, J=8.9 Hz), 7.79 (1H, d, J=6.2 Hz), 7.59 (1H, d, J=8.9 Hz), 7.48 (1H, s), 7.42 (1H, d, J=6.2 Hz), 4.63 (1H, br s), 4.42—4.31 (1H, m), 4.10—3.98 (1H, m), 3.96 (3H, s), 3.96—3.27 (3H, m), 2.48—2.37 (1H, m), 2.28—2.13 (1H, m), 2.08—1.92 (2H, m). MS m/z: 269 (M^+), 175.

1-((8a*S*)-Octahydropyrrolo[1,2-*a*]pyrazin-2-yl)-7-methoxyisoquinoline (10i) This compound was synthesized using the same procedure as for **15a** starting with 1-chloro-7-methoxyisoquinoline and (8a*S*)-octahydropyrrolo-[1,2-*a*]pyrazine. ^{1}H-NMR ($CDCl_3$) δ: 8.07 (1H, d, J=5.4 Hz), 7.68 (1H, d, J=8.9 Hz), 7.40 (1H, d, J=2.7 Hz), 7.29 (1H, dd, J=2.3, 8.9 Hz), 7.21 (1H, d, J=5.4 Hz), 3.94 (3H, s), 3.87—3.84 (1H, m), 3.79—3.75 (2H, m), 3.22—3.16 (2H, m), 2.93—2.88 (1H, m), 2.64—2.58 (1H, m), 2.40—2.38 (1H, m), 2.29 (1H, q, J=8.5 Hz), 1.94—1.76 (3H, m), 1.65—1.52 (1H, m). MS m/z: 283 (M^+), 96.

1-((8a*R*)-Octahydropyrrolo[1,2-*a*]pyrazin-2-yl)-7-methoxyisoquinoline (10j) This compound was synthesized using the same procedure as for **15a** starting with 1-chloro-7-methoxyisoquinoline and (8a*R*)-octahydropyrrolo[1,2-*a*]pyrazine. ^{1}H-NMR ($CDCl_3$) δ: 8.06 (1H, d, J=5.8 Hz), 7.66 (1H, dd, J=3.4, 8.9 Hz), 7.39 (1H, d, J=2.3 Hz), 7.27 (1H, dd, J=2.3, 8.9 Hz), 7.19 (1H, d, J=5.4 Hz), 3.93 (3H, s), 3.85 (1H, td, J=2.3, 12.0 Hz), 3.77—3.74 (1H, m), 3.20—3.15 (3H, m), 2.89 (1H, t, J=10.8 Hz), 2.60 (1H, t, J=11.2 Hz), 2.39—2.37 (1H, m), 2.32—2.25 (1H, m), 1.93—1.83 (2H, m), 1.80—1.76 (1H, m), 1.59—1.51 (1H, m). MS m/z: 283 (M^+), 96.

7-Methoxy-1-octahydropyrido[1,2-*a*]pyrazin-2-ylisoquinoline (10k) This compound was synthesized using the same procedure as for **15b** starting with 1-chloro-7-methoxyisoquinoline and octahydropyrido[1,2-*a*]-pyrazine. ^{1}H-NMR ($CDCl_3$) δ: 8.07 (1H, d, J=5.4 Hz), 7.67 (1H, d, J=8.9 Hz), 7.40 (1H, d, J=2.7 Hz), 7.28 (1H, dd, J=2.7, 8.9 Hz), 7.20 (1H, d, J=5.8 Hz), 3.94 (3H, s), 3.72 (1H, qd, J=2.7, 12.3 Hz), 3.57 (1H, td, J=2.7, 12.3 Hz), 3.26—3.16 (1H, m), 2.98—2.84 (3H, m), 2.63 (1H, dt, J=2.7, 11.6 Hz), 2.35—2.25 (1H, m), 2.25—2.15 (1H, m), 1.84—1.54 (4H, m), 1.42—1.32 (2H, m). MS m/z: 297 (M^+), 110.

7-(4-Methylpiperazin-1-yl)furo[2,3-*c*]pyridine (15b) This compound was synthesized using the same procedure as for **15a** starting with 7-chlorofuro[2,3-*c*]pyridine and 1-methylpiperazine. ^{1}H-NMR ($CDCl_3$) δ: 7.95 (1H, d, J=5.3 Hz), 7.62 (1H, d, J=1.9 Hz), 6.96 (1H, d, J=5.4 Hz), 6.72 (1H, d, J=1.9 Hz), 3.88 (4H, t, J=5.0 Hz), 2.59 (4H, t, J=5.0 Hz), 2.38 (3H, s). MS m/z: 217 (M^+), 147.

7-Piperazin-1-ylthieno[2,3-*c*]pyridine (15c) This compound was synthesized using the same procedure as for **15a** starting with 7-chlorothieno[2,3-*c*]pyridine and anhydrous piperazine. ^{1}H-NMR ($CDCl_3$) δ: 8.14

(1H, d, J=5.8 Hz), 7.58 (1H, d, J=5.4 Hz), 7.32 (1H, d, J=5.4 Hz), 7.25 (1H, d, J=5.4 Hz), 3.64 (4H, t, J=5.0 Hz), 3.09 (4H, t, J=5.0 Hz). MS m/z: 219 (M^+), 151.

7-(4-Methylpiperazin-1-yl)thieno[2,3-*c*]pyridine (15d) This compound was synthesized using the same procedure as for **15a** starting with 7-chlorothieno[2,3-*c*]pyridine and 1-methylpiperazine. ^{1}H-NMR ($CDCl_3$) δ: 8.13 (1H, d, J=5.8 Hz), 7.57 (1H, d, J=5.4 Hz), 7.31 (1H, d, J=5.4 Hz), 7.24 (1H, d, J=5.4 Hz), 3.71 (4H, t, J=5.0 Hz), 2.63 (4H, t, J=5.0 Hz), 2.38 (3H, s). MS m/z: 233 (M^+), 163.

4-Piperazin-1-ylfuro[3,2-*c*]pyridine (16a) This compound was synthesized using the same procedure as for **15a** starting with 4-chlorofuro[3,2-*c*]pyridine and anhydrous piperazine. ^{1}H-NMR ($CDCl_3$) δ: 8.05 (1H, d, J=5.8 Hz), 7.53 (1H, d, J=2.0 Hz), 6.94 (1H, dd, J=0.8, 5.8 Hz), 6.81 (1H, dd, J=1.2, 2.3 Hz), 3.67(4H, t, J=5.0 Hz), 3.05 (4H, t, J=5.0 Hz). MS m/z: 203 (M^+), 135.

4-(4-Methylpiperazin-1-yl)furo[3,2-*c*]pyridine (16b) This compound was synthesized using the same procedure as for **15a** starting with 4-chlorofuro[3,2-*c*]pyridine and 4-methylpiperazine. ^{1}H-NMR ($CDCl_3$) δ: 8.05 (1H, d, J=5.8 Hz), 7.53 (1H, d, J=2.3 Hz), 6.94 (1H, dd, J=1.2, 5.8 Hz), 6.81 (1H, dd, J=1.2, 2.3 Hz), 3.74 (4H, t, J=5.0 Hz), 2.59 (4H, t, J=5.0 Hz), 2.37 (3H, s). MS m/z: 217 (M^+), 147.

4-Piperazin-1-ylthieno[3,2-*c*]pyridine (16c) This compound was synthesized using the same procedure as for **15a** starting with 4-chlorothieno[3,2-*c*]pyridine and anhydrous piperazine. ^{1}H-NMR ($CDCl_3$) δ: 8.09 (1H, d, J=5.8 Hz), 7.42—7.35 (3H, m), 3.50 (4H, t, J=5.0 Hz), 3.11 (4H, t, J=5.0 Hz). MS m/z: 219 (M^+), 151.

4-(4-Methylpiperazin-1-yl)thieno[3,2-*c*]pyridine (16d) This compound was synthesized using the same procedure as for **15a** starting with 4-chlorothieno[3,2-*c*]pyridine and 1-methylpiperazine. ^{1}H-NMR ($CDCl_3$) δ: 8.08 (1H, d, J=5.8 Hz), 7.41—7.33 (3H, m), 3.57 (4H, t, J=5.0 Hz), 2.64 (4H, t, J=5.0 Hz), 2.39 (3H, s). MS m/z: 233 (M^+), 163.

7-((8a*S*)-Octahydropyrrolo[1,2-*a*]pyrazin-2-yl)furo[2,3-*c*]pyridine (17a=TZB-20810) This compound was synthesized using the same procedure as for **15a** starting with 7-chlorofuro[2,3-*c*]pyridine and (8a*S*)-octahydropyrrolo[1,2-*a*]pyrazine. ^{1}H-NMR ($CDCl_3$) δ: 7.95 (1H, d, J=5.4 Hz), 7.63 (1H, d, J=2.3 Hz), 6.95 (1H, d, J=5.4 Hz), 6.72 (1H, d, J=1.9 Hz), 4.80—4.76 (1H, m), 4.71—4.66 (1H, m), 3.33—3.14 (3H, m), 2.87—2.81 (1H, m), 2.44—2.40 (1H, m), 2.38—2.13 (2H, m), 1.96—1.84 (2H, m), 1.82—1.74 (1H, m), 1.56—1.51 (1H, m). MS m/z: 243 (M^+), 147.

7-((8a*R*)-Octahydropyrrolo[1,2-*a*]pyrazin-2-yl)furo[2,3-*c*]pyridine (17b) This compound was synthesized using the same procedure as for **15a** starting with 7-chlorofuro[2,3-*c*]pyridine and (8a*R*)-octahydropyrrolo[1,2-*a*]pyrazine. ^{1}H-NMR ($CDCl_3$) δ: 7.93 (1H, d, J=5.0 Hz), 7.62 (1H, d, J=1.9 Hz), 6.94 (1H, d, J=5.4 Hz), 6.70 (1H, d, J=2.3 Hz), 4.79—4.74 (1H, m), 4.69—4.65 (1H, m), 3.32—3.12 (3H, m), 2.86—2.80 (1H, m), 2.43—2.39 (1H, m), 2.37—2.12 (2H, m), 1.95—1.83 (2H, m), 1.81—1.70 (1H, m), 1.56—1.49 (1H m,). MS m/z: 243 (M^+), 147.

7-((8a*S*)-Octahydropyrrolo[1,2-*a*]pyrazin-2-yl)thieno[2,3-*c*]pyridine (17c) This compound was synthesized using the same procedure as for **15a** starting with 7-chlorothieno[2,3-*c*]pyridine and (8a*S*)-octahydropyrrolo[1,2-*a*]pyrazine. ^{1}H-NMR ($CDCl_3$) δ: 8.12 (1H, d, J=5.4 Hz), 7.57 (1H, d, J=5.4 Hz), 7.31 (1H, d, J=5.4 Hz), 7.23 (1H, d, J=5.4 Hz), 4.44—4.40 (1H, m), 4.34—4.31 (1H, m), 3.27—3.26 (1H, m), 3.23—3.11 (2H, m), 2.92—2.86 (1H, m), 2.50—2.45 (1H, m), 2.27—2.21 (2H, m), 1.95—1.86 (2H, m), 1.83—1.76 (1H, m), 1.59—1.51 (1H, m). MS m/z: 259 (M^+), 163.

7-((8a*R*)-Octahydropyrrolo[1,2-*a*]pyrazin-2-yl)thieno[2,3-*c*]pyridine (17d) This compound was synthesized using the same procedure as for **15a** starting with 7-chlorothieno[2,3-*c*]pyridine and (8a*R*)-octahydropyrrolo[1,2-*a*]pyrazine. ^{1}H-NMR ($CDCl_3$) δ: 8.13 (1H, d, J=5.4 Hz), 7.57 (1H, d, J=5.4 Hz), 7.31 (1H, d, J=5.4 Hz), 7.24 (1H, d, J=5.4 Hz), 4.41 (1H, td, J=2.3, 12.0 Hz), 4.35—4.31 (1H, m), 3.24—3.15 (3H, m), 2.93—2.87 (1H, m), 2.51—2.45 (1H, m), 2.28—2.22 (2H, m), 1.94—1.87 (2H, m), 1.81—1.73 (1H, m), 1.56—1.52 (1H, m). MS m/z: 259 (M^+), 163.

4-((8a*S*)-Octahydropyrrolo[1,2-*a*]pyrazin-2-yl)furo[3,2-*c*]pyridine (18a) This compound was synthesized using the same procedure as for **15a** starting with 4-chlorofuro[3,2-*c*]pyridine and (8a*S*)-octahydropyrrolo[1,2-*a*]pyrazine. ^{1}H-NMR ($CDCl_3$) δ: 8.04 (1H, d, J=5.8 Hz), 7.53 (1H, d, J=2.3 Hz), 6.93 (1H, d, J=5.8 Hz), 6.83 (1H, dd, J=0.8, 2.3 Hz), 4.48 (1H, ddd, J=1.9, 2.7, 12.3 Hz), 4.35—4.31 (1H, m), 3.30—3.23 (1H, m), 3.18—3.13 (2H, m), 2.91—2.85 (1H, m), 2.44—2.38 (1H, m), 2.25—2.13 (2H, m), 1.95—1.84 (2H, m), 1.82—1.50 (2H, m). MS m/z: 243 (M^+), 147.

4-((8a*R*)-Octahydropyrrolo[1,2-*a*]pyrazin-2-yl)furo[3,2-c]pyridine (18b) This compound was synthesized using the same procedure as for **15a** starting with 4-chlorofuro[3,2-*c*]pyridine and (8a*R*)-octahydropyrrolo[1,2-*a*]pyrazine. ^{1}H-NMR ($CDCl_3$) δ: 8.04 (1H, d, J=5.8 Hz), 7.53 (1H, d, J=2.3 Hz), 6.93 (1H, dd, J= 0.8, 5.8 Hz), 6.83—6.82 (1H, m), 4.50—4.46 (1H, m), 4.35—4.31 (1H, m), 3.27 (1H, dt, J=3.1, 12.3 Hz), 3.18—3.14 (2H, m), 2.92—2.86 (1H, m), 2.41 (1H, dt, J=3.4, 11.2 Hz), 2.25—2.15 (2H, m), 1.95—1.85 (2H, m), 1.82—1.75 (1H, m), 1.56—1.48 (1H, m). MS m/z: 243 (M^+), 147.

4-((8a*S*)-Octahydropyrrolo[1,2-*a*]pyrazin-2-yl)thieno[3,2-*c*]pyridine (18c) This compound was synthesized using the same procedure as for **15a** starting with 4-chlorothieno[3,2-*c*]pyridine and (8a*S*)-octahydropyrrolo[1,2-*a*]pyrazine. ^{1}H-NMR ($CDCl_3$) δ: 8.04 (1H, d, J=5.4 Hz), 7.43—7.29 (3H, m), 4.17—4.12 (1H, m), 4.07—4.02 (1H, m), 3.28—3.22 (1H, m), 3.19—3.14 (2H, m), 2.94—2.88 (1H, m), 2.51 (1H, dt, J=2.7, 7.2 Hz), 2.29—2.22 (2H, m), 1.94—1.85 (2H, m), 1.85—1.75 (1H, m), 1.56—1.50 (1H, m). MS m/z: 259 (M^+), 107.

4-((8a*R*)-Octahydropyrrolo[1,2-*a*]pyrazin-2-yl)thieno[3,2-*c*]pyridine (18d) This compound was synthesized using the same procedure as for **15a** starting with 4-chlorothieno[3,2-*c*]pyridine and (8a*R*)-octahydropyrrolo[1,2-*a*]pyrazine. ^{1}H-NMR ($CDCl_3$) δ: 8.07 (1H, d, J=5.4 Hz), 7.43—7.41 (1H, m), 7.37 (1H, d, J=5.4 Hz), 7.33 (1H, dd, J=0.8, 5.8 Hz), 4.17—4.13 (1H, m), 4.05 (1H, ddd, J=1.9, 5.0, 12.7 Hz), 3.26 (1H, dt, J=2.7, 12.3 Hz), 3.19—3.15 (2H, m), 2.95—2.89 (1H, m), 2.52 (1H, dt, J=2.7, 11.2 Hz), 2.30—2.23 (2H, m), 1.94—1.87 (2H, m), 1.82—1.76 (1H, m), 1.56—1.49 (1H, m). MS m/z: 259 (M^+), 163.

4-((8a*S*)-Octahydropyrrolo[1,2-*a*]pyrazin-2-yl)-2-methylthieno[3,2-*c*]pyridine (18e) This compound was synthesized using the same procedure as for **15a** starting with 4-chloro-2-methylthieno[3,2-*c*]pyridine and (8a*S*)-octahydropyrrolo[1,2-*a*]pyrazine. ^{1}H-NMR ($CDCl_3$) δ: 8.01 (1H, d, J=5.4 Hz), 7.23—7.22 (1H, m), 7.03 (1H, d, J=1.2 Hz), 4.07 (1H, td, J=2.3, 12.0 Hz), 3.98 (1H, d, J=11.8 Hz), 3.23—3.08 (3H, m), 2.90—2.84 (1H, m), 2.58 (3H, dd, J=1.2, 4.3 Hz), 2.53—2.47 (2H, m), 2.37—2.27 (2H, m), 1.93—1.85 (2H, m), 1.81—1.75 (1H, m), 1.54—1.50 (1H, m). MS m/z: 273 (M^+), 177.

7-Octahydropyrido[1,2-*a*]pyrazin-2-ylfuro[2,3-*c*]pyridine (19a) This compound was synthesized using the same procedure as for **15a** starting with 7-chlorofuro[2,3-*c*]pyridine and octahydropyrido[1,2-*a*]pyrazine. ^{1}H-NMR ($CDCl_3$) δ: 7.95 (1H, d, J=5.4 Hz), 7.62 (1H, d, J=2.3 Hz), 6.95 (1H, d, J=5.4 Hz), 6.71 (1H, d, J=2.3 Hz), 4.65—4.58 (1H, m), 4.49—4.43 (1H, m), 3.30—3.20 (1H, m), 2.94—2.77 (3H, m), 2.47—2.38 (1H, m), 2.15—2.04 (2H, m), 1.87—1.77 (1H, m), 1.73—1.64 (3H, m), 1.40—1.29 (2H, m). MS m/z: 257 (M^+), 110.

7-Octahydropyrido[1,2-*a*]pyrazin-2-ylthieno[2,3-*c*]pyridine (19b) This compound was synthesized using the same procedure as for **15a** starting with 7-chlorothieno[2,3-*c*]pyridine and octahydropyrido[1,2-*a*]pyrazine. ^{1}H-NMR ($CDCl_3$) δ: 8.12 (1H, d, J=5.4 Hz), 7.57 (1H, d, J=5.4 Hz), 7.31 (1H, d, J=5.4 Hz), 7.23 (1H, d, J=5.4 Hz), 4.32—4.24 (1H, m), 4.16—4.09 (1H, m), 3.26 (1H, dt, J=2.7, 12.3 Hz), 2.95—2.82 (3H, m), 2.49 (1H, dt, J=3.1, 12.0 Hz), 2.20—2.09 (2H, m), 1.86—1.78 (1H, m), 1.74—1.60 (3H, m), 1.40—1.31 (2H, m). MS m/z: 273 (M^+), 110.

Evaluation of Biological Activity.[13,14] Affinity Measurement of the Compounds to Human 5-HT$_{1A}$ Receptor (*in Vitro*) CHO cell membrane sample in which human 5-HT$_{1A}$ receptor was expressed 0.25 ml (about 50 units) was added to 24.75 ml of incubation buffer solution A (an aqueous solution of a mixture of 50 mM of Tris–hydrochloric acid, 10 mM of magnesium sulfate, 0.5 mM of EDTA and 0.1% ascorbic acid, whose pH was adjusted to 7.4 at 27 °C with 1 N-aqueous sodium hydroxide solution), and labeled as membrane sample suspension A. Separately, each test compound was made into 270 μM DMSO solution and diluted to a prescribed concentration with the incubation buffer solution A, to provide a compound solution. A piece of polypropylene tube was charged with 20 μl of [^{3}H]8-OH-DPAT (the concentration of the [^{3}H]8-OH-DPAT had been advancedly adjusted to render its concentration in the reaction mixture to 0.25 nM) and 20 μl of one of the compound solutions. Further 500 μl of the membrane sample suspension A was added into the tube, followed by 60 minutes' incubation at 27 °C. The reaction was terminated by rapid filtration of the reaction mixture through GF/C filter which had been advancedly immersed in a solution formed by adding to the incubation buffer solution A polyethyleneimine to a concentration of 0.3%, using Brandel cell harvester. Then the filter was washed with about 5 ml of 50 mM of Tris–hydrochloric acid which had been cooled to 4 °C. The filter was once again washed after similar operation. Residual radioactivity on the filter was measured with liquid scintillation counter (Aloka Co., LSC-5100), and percent inhibition (%) of each test compound to binding of [^{3}H]-8-OH-DPAT to 5-HT$_{1A}$ receptor at a concentration of 0.25 nM was calculated.

Measurement of Affinity of the Compounds to Human 5-HT$_3$ Recep-

tor (*in Vitro*) HEK-293 cell membrane sample in which human 5-HT_3 receptor was expressed (purchased from BIOLINKS K.K.) 0.05 ml (about 50 microassay) was added to 24.95 ml of incubation buffer solution B (an aqueous solution of a mixture of 50 mM of Tris–hydrochloric acid, 5 mM of magnesium chloride and 1 mM of EDTA, whose pH was adjusted to 7.5 at 25 °C with 1 N-aq. NaOH) and homogenized, to provide a membrane sample suspension B. Separately, each test compound was made into 270 μM of DMSO solution and diluted to a prescribed concentration with the incubation buffer solution B to provide a compound solution. A piece of polypropylene tube was charged with 20 μl of [^{3}H]BRL-43694 (the concentration of [^{3}H]BRL-43694 had been advancedly adjusted to render its concentration in the reaction mixture to 0.5 nM) and 20 μl of one of the compound solutions. Further 500 μl of the membrane sample suspension B was added into the tube, followed by 60 minutes' incubation at 25 °C. The reaction was terminated by rapid filtration of the reaction mixture through GF/B filter which had been advancedly immersed in a solution formed by adding to the incubation buffer solution B polyethyleneimine to a concentration of 0.5%, using Brandel cell harvester. Then the filter was washed with about 5 ml of 50 mmol/l of Tris–hydrochloric acid which had been cooled to 4 °C. The filter was once again washed after similar operation. Residual radioactivity on the filter was measured with liquid scintillation counter (Aloka Co., LSC-5100), and percent inhibition (%) of each test compound to binding of [^{3}H]BRL-43694 to 5-HT_3 receptor at a concentration of 0.5 nmol/l were calculated.

5-HT_{1A} Agonist-Induced [^{35}S]GTPγS Binding Assays[16] Human 5-HT_{1A} receptor (cloned human serotonin receptor subtype 1A (h5HT_{1A}), produced in CHO cells, PerkinElmer, Inc.) was thawed on ice and diluted with incubation buffer (20 mM HEPES/3 mM $MgCl_2$/120 mM NaCl, pH 7.4 at 30 °C). Membranes were incubated with GDP (20 μM) and the test drugs for 20 min at 30 °C in a volume of 900 μl and then placed on ice for 15 min. [^{35}S]GTPγS (100 pM) was added to all tubes to yield a final volume of 1 ml and the tubes were further incubated for 30 min at 30 °C. Incubation was terminated by filtering the mixtures through GF/B filters using a Brandel cell harvester. The filters were washed twice with 5 ml of cold wash buffer (20 mM HEPES/3 mM $MgCl_2$, pH 7.4 at 4 °C). Radioactivity retained on the filters was counted by a liquid scintillation counter (Aloka Co., LSC-5100).

Inhibition of 5-HT_3 Receptor Mediated Contraction in Guinea-Pig Ileum[17] The ileum preparations were suspended in an organ bath containing Tyrode solution (137 mM NaCl/3 mM KCl/2 mM $CaCl_2$/1 mM $MgCl_2$/12 mM $NaHCO_3$/0.4 mM NaH_2PO_4/6 mM D-(+)-glucose), warmed to 37 °C and aerated with a mixture of 5% CO_2 and 95% O_2. Isotonic contractions under a loading tension of 1 g were recorded using an isotonic force transducer. Experiments were started after stable contractions induced by 10 μM 2-methyl-5-HT were obtained at least 3 times. The vehicle (DMSO) or the test drugs were added to the organ bath and the preparations were exposed to the vehicle or test drugs for 20 min. Then 2-methyl-5-HT (10 μM) was added to the organ bath and the contractions were recorded.

5-HT_{1A} Receptor-Mediated Behavior and Hypothermia in Rats Rats were acclimated to the test environment for 2 weeks prior to testing and conditioned to the test procedures during this period. On the day of the experiment, rats were acclimated to the test cage for 1 h. Test compound or vehicle (saline containing diluted hydrochloric acid) was injected intraperitoneally, and then 5-HT_{1A} receptor-mediated behavior (lower lip retraction and flat body posture) was measured in the test cage. Behavioral responses were measured at 5, 10, 20 and 30 min after administration using a 0—3 scale as previously described in the literature.[18] The rectal temperature was recorded before and at 30 min after administration of the test drugs using a thermistoprobe that was inserted into the rectum, 3 cm from the anal orifice. The difference between the temperatures measured before and after administration was designated as the index of hypothermia.

5-HT-Induced Bradycardia (von Bezold–Jarisch Reflex) in Rats The surgical procedures and mean heart rate recordings were performed as follows: animals were anesthetized with urethane at a dose of 1.25 g/kg, i.p. and then polyethylene cannulas were inserted into the right common carotid artery and vein to measure the blood pressure and to administer 5-HT, respectively. The duodenum was incised, decorticated 2—3 cm from the stomach and cannulated for intraduodenal administration of the test drugs. Blood pressure was monitored using a pressure amplifier (AP-601G; Nihon Kohden Co., Tokyo) and the mean heart rate was recorded by a tachometer (AT-601G; Nihon Kohden Co., Tokyo) triggered by blood pressure pulsation. 5-HT was injected intravenously at 300 μg/kg to evoke a transient bradycardia (BJ reflex). Following recovery to normal blood pressure and heart rate, the test drugs and vehicle (0.5% Tween 80) were administered into the duodenum. Thirty minutes later, 5-HT was re-administered intravenously and the bradycardia was assessed.

References

1) Talley N. J., Zinsmeister A. R., Van Dyke C., Melton L. J., *Gastroenterology*, **101**, 923—934 (1991).
2) Drossman D. A., Li Z., Andruzzi E., Temple R. D., Talley N. J., Thompson W. G., Whitehead W. E., Janssens J., Funch-Jensen P., Corazziari E., Richter J. E., Koch G. G., *Dig. Dis. Sci.*, **38**, 1569—1580 (1993).
3) Whitehead W. E., Crowell M. D., *Gastroenterol. Clin. North Am.*, **20**, 249—267 (1991).
4) Camilleri M., Choi M.-G., *Aliment. Pharmacol. Ther.*, **11**, 3—15 (1997).
5) Morreale A., Gálvez-Ruano E., Iriepa-Canalda I., Boyd B. D., *J. Med. Chem.*, **41**, 2029—2039 (1998).
6) Camilleri M., Northcutt A. R., Kong S., Duke G., McSorley D., Mangel A., *Lancet*, **355**, 1035—1040 (2000).
7) Tchao P., Béatrice L. G., Daniel-Henri C., Gérard A., Bruno P., Pierre R., Gérald G., *J. Med. Chem.*, **37**, 1779—1793 (1994).
8) Milligana G., Kelletta E., Dacquetb C., Dubreuilb V., Jacobyb E., Millana M. J., Lavielleb G., Spedding M., *Neuropharmacology*, **40**, 334—344 (2001).
9) Hori M., Suzuki K., Yamamoto T., Nakajima F., Ozaki A., Ohtaka H., *Chem. Pharm. Bull.*, **41**, 1832—1841 (1993).
10) Campiani G., Cappelli A., Nacci V., Anzin M., Vomero S., Hamon M., Cagnotto A., Fracasso C., Uboldi C., Caccia S., Consolo S., Mennini T., *J. Med. Chem.*, **40**, 3670—3678 (1997).
11) New J. S., Christopher W. L., Yevich J. P., Butler R., Schlemmer R. F., VanderMaelen C. P., Cipollina J. A., *J. Med. Chem.*, **32**, 1147—1156 (1989).
12) Botre C., Botre F., Jommi G., Signorini R., *J. Med. Chem.*, **29**, 1814—1820 (1986).
13) Tamaoki S., Yamauchi Y., Nakano Y., Sakano S., Asagarasu A., Sato M., *J. Pharmacol. Exp. Ther.*, **322**, 1315—1323 (2007).
14) Sato M., Matsui T., Asagarasu A., Hayashi H., Araki S., Tamaoki S., Takahashi N., Yamauchi Y., Yamamoto Y., Yamamoto N., Ogawa C., WO2005082887 (A1).
15) This examination was done in MDS pharma Service (www.mdsps.com).
16) Stanton J. A., Beer M. S., *Eur. J. Pharmacol.*, **320**, 267—275 (1997).
17) Butler A., Elswood C. J., Burridge J., Ireland S. J., Bunce K. T., Kilpatrick G. J., Tyers M.B., *Br. J. Pharmacol.* **101**, 591—598 (1990).
18) Smith L. M., Peroutka S. J., *Pharmacol. Biochem. Behav.*, **24**, 1513—1519 (1986).