(R)-1,2,3,4-Tetrahydro[1]benzothieno[2,3-c]pyridines: Novel Optically Active Compounds with Strong 5-HT_{1A} Receptor Binding Ability Exhibiting Anticonflict Activity and Lessening of Memory Impairment†

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(R)-1,2,3,4-Tetrahydro[1]benzothieno[2,3-c]pyridine derivatives (**60-114**) were synthesized. The (R)-isomers have affinity for the 5-HT_{1A} receptor while the (S)-isomers have no such ability. The affinity of the (R)-isomers was discussed on the basis of structure–activity relationships between the affinity and hydrophobicity of the (R)-isomers. Compounds **71** and **107**, which are representative derivative compounds, have anticonflict activity and lessening of memory impairment. In particular, compound **107** cannot bind to receptors other than the 5-HT_{1A} receptor, demonstrating that it is a unique compound with a different mechanism of action from that of conventional anxiolytics.

Introduction

The neurotransmitter 5-HT has recently received much attention. 5-HT is involved in numerous physiological (e.g. hemodynamics, feeding, sleeping) and pathophysiological (e.g. depression, hypertension, migraine anxiety) processes and interacts with various distinct membrane receptors. Buspirone[1] is widely used as an anxiolytic which binds to the 5-HT_{1A} receptor. Tandospirone[2] is now under clinical trial. However, since these drugs also bind to the DA_2 receptor,[3] it is difficult to explain the mechanisms of their anticonflict effect only by their affinity for the 5-HT_{1A} receptor. We reported on racemic 1,2,3,4-tetrahydro[1]-benzothieno[2,3-c]pyridine derivatives[4] with selective affinity for the 5-HT_{1A} receptor.

In this paper, we report that only (R)-isomers of 1,2,3,4-tetrahydro[1]benzothieno[2,3-c]pyridines can bind selectively to the 5-HT_{1A} receptor, while their (S)-isomers[5] cannot. We describe the synthesis of optically active 1,2,3,4-tetrahydro[1]benzothieno[2,3-c]pyridines as well as their anticonflict activities, lessening of memory impairments, and structure–activity relationships.

Chemistry

We synthesized novel 2- or 3-substituted (R)-1,2,3,4-tetrahydro[1]benzothieno[2,3-c]pyridines (**60–114**) as shown in Scheme I. The optical resolution was the most important step in our synthetic routes. (R)-Thiotryptophan **2** (99.7% *ee*) was obtained by deacetylation[6] of **1**[7] with Acylase.[8] The ester **3** was synthesized from **2** using the standard procedure.[9,10] (R)-1,2,3,4-Tetrahydro[1]-benzothieno[2,3-c]pyridine-3-carboxylic acid methyl ester **4** was obtained from **3** according to the standard procedure of Pictet–Spengler isoquinoline synthesis.[11-13] The reaction[14] of **4** with $(Boc)_2O/NEt_3$ gave **5**, which was hydrolyzed with NaOH to give (R)-*N*-Boc acid **6** (91%) and (S)-*N*-Boc acid **7** (9%). Treatment[15] of a mixture **6** and **7** with D-(+)-1-phenylethylamine in *i*-PrOH yielded recovered **7** and D-(+)-1-phenylethylamine salt of **6** which was acidified with HCl to give **6**. The acid **6** was converted to **8–59** by reaction with various amines and alcohols in the presence of DPPA/NEt_3. The compounds **60–111** were obtained by treatment of **8–59** with 1 N HCl in AcOEt. The acid **112** was obtained by treatment of **6** with 1 N HCl in AcOEt. The compounds (**113–114**) were synthesized by the reaction of **63** with MeI, *p*-methylbenzoyl chloride, and NEt_3 in $CHCl_3$.

Pharmacological Methods

Affinity for the 5-HT_{1A} receptor[16] was measured by use of [^{3}H]-8-OH-DPAT and receptor preparation obtained from hippocampus of male Sprague–Dawley rats. The procedure is described in the Experimental Section.

Anticonflict activity was evaluated by a water lick conflict test[17] using groups of male Wistar rats. The procedure is described in the Experimental Section.

Lessening of memory impairment was evaluated by a passive avoidance test[18,19] using groups of male ddy mice. The procedure is described in the Experimental Section.

Result and Discussion

1. **Affinity of Optically Active 1,2,3,4-Tetrahydro-[1]benzothieno[2,3-c]pyridines for the 5-HT_{1A} Receptor.** The affinity of the (R)-compounds for the 5-HT_{1A} receptor is shown as the inhibition percentage in Tables I and II. The large difference in affinity for the 5-HT_{1A} receptor between (R)- and (S)-isomers is shown in typical cases with **63** and **107** (Table I); the IC_{50} value of (S)-isomer of **63** or **107** is more than 184- and 94-times that of **63** or **107**. IC_{50} (nM) values for other compounds which showed more than 65% inhibition are also listed in Table I. These compounds have strong affinity of the order of 10-100 (nM).

2. **Relationship between Hydrophobicity of the Compound and Affinity for the 5-HT_{1A} Receptor.** The retention time (t_r) of the compound on reversed-phase HPLC (Table I) was employed as a measure of the hydrophobicity.[20] The affinity for the 5-HT_{1A} receptor (Table I) was plotted against the hydrophobicity (log t_r) as shown in Figure 1. A good correlation was observed up

† Abbreviations: 5-HT, serotonin; DA, dopamine; BZP, benzodiazepine; HPLC, high-performance liquid chromatography; $(Boc)_2O$, di-*tert*-butyl dicarbonate; DPPA, diphenyl phosphorazidate.

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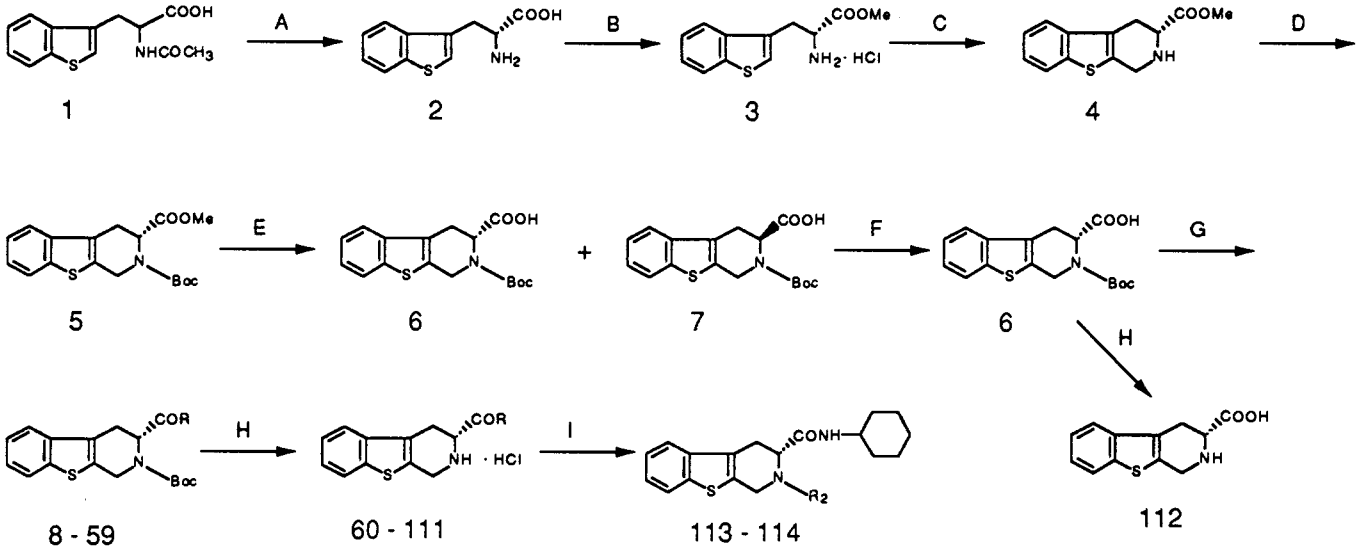
Scheme I[a]



[a] (A) Acylase; (B) $SOCl_2$/MeOH; (C) 30% HCHO/EtOH–H_2O; (D) $(Boc)_2O/NEt_3$; (E) NaOH/H^+; (F) D-(+)-$PhCH(CH_3)NH_2$; (G) RH/DPPA/NEt_3/DMF; (H) 1 N HCl/AcOEt; (I) CH_3I or *p*-methylbenzoyl chloride/NEt_3 (R = HNC_6H_{11}).

to the hydrophobicity of 1.3. But the hydrophobicity is more than 1.3 in the case of compounds **105** and **106**, with resulting decreased affinity for the 5-HT_{1A} receptor. Compounds **75** and **87–93** without an NH group showed no affinity, probably because of their tertiary amide structure (Table I).

The result indicates that large molecules can bind to the receptor and that the affinity for the 5-HT_{1A} receptor increases as the molecular size increases. Thus it is concluded that the presence of (*R*)-1,2,3,4-tetrahydro[1]-benzothieno[2,3-*c*]pyridine skeleton and the attachment of an appropriate hydrophobic group are necessary for the good affinity for the 5-HT_{1A} receptor.

3. Anticonflict Activity and Lessening of Memory Impairment with Representative Compounds 71 and 107. The most active compounds **71** and **107** were examined following oral administration for anticonflict activity in the water lick conflict test where they were as potent as diazepam (Table III). In addition, the compounds **71** and **107** was also tested with respect to the ability to reverse scopolamine-induced amnesia in a passive avoidance test used to assess memory impairment. While the compounds were active and increased latency over that seen with diazepam or scopolamine, they did not return latency to that seen in control animals.

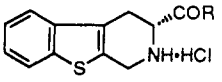
4. Binding Ability of Compound 107 to Various Receptors. Binding ability of compound **107** to various receptors other than 5-HT_{1A} were in order BZP ($>1.0 \times 10^{-4}$ M), $GABA_B$ ($>1.0 \times 10^{-4}$ M), DA_1 (3.0×10^{-5} M), DA_2 ($>1.0 \times 10^{-4}$ M), α_1 (6.0×10^{-6} M), α_2 (1.0×0^{-4} M), β_1 ($>1.0 \times 10^{-4}$ M), β_2 ($>1.0 \times 10^{-4}$ M), 5-HT_2 ($>1.0 \times 10^{-4}$ M), 5-HT_3 ($>1.0 \times 10^{-4}$ M). Compound **107** bound selectively to the 5-HT_{1A} ($(5.31 \pm 0.07) \times 10^{-8}$ M) receptor in Table IV. On the other hand, both tandospirone ($DA_2 = 2.8 \times 10^{-6}$ M[3], 5-$HT_{1A} = 2.7 \times 10^{-8}$ M), and buspirone ($DA_2 = 9.0 \times 10^{-8}$ M[3], 5-$HT_{1A} = 1.5 \times 10^{-8}$ M) also bound to the DA_2 receptor in Table IV, so that mechanism of action cannot be explained just from the 5-HT_{1A} receptor binding. Indeed drug discrimination assay systems are widely used for characterization of drugs. However, this procedure is usually employed to compare the so called "discriminative stimulus effect" of the drug. It is well-known that this discriminative stimulus does not always reflect the efficacy of the drug. In addition, in our preliminary data compound **107** seems not to be a simple agonist for the 5-HT_{1A} receptor different from 8-OH-DPAT. Discriminative studies are in progress to characterize compound **107** as a 5-HT_{1A} ligand; however, we suspect that a drug discrimination assay is not adequate for discussing whether compound **107** is an agonist or an antagonist for the 5-HT_{1A} receptor. From current pharmacological studies, compound **107** clearly inhibited forskolin-stimulated adenylate cyclase activity in rat hippocampal membrane through the 5-HT_{1A} receptor similar to the inhibitory effect of typical 5-HT_{1A} agonist (e.g. 8-OH-DPAT, tandospirone, buspirone). The potency seems to be weaker compared with 8-OH–DPAT. Moreover the anticonflict effect of compound **107** was clearly antagonized by NAN-190,5-HT_{1A} antagonist. Therefore presently we suspect compound **107** is an agonist or a partial agonist for the postsynaptic 5-HT_{1A} receptor. Therefore, it was concluded that compound **107** is a unique compound which selectively binds to the 5-HT_{1A} receptor and exhibits anticonflict activity and lessening of memory impairment.

Conclusion

(*R*)-1,2,3,4-Tetrahydro[1]benzothieno[2,3-*c*]pyridine derivatives (**60–114**) were synthesized. The (*R*)-isomers have affinity for the 5-HT_{1A} receptor while the (*S*)-isomers have no such ability. The large (*R*)-isomer can bind to the receptor, and the affinity for the 5-HT_{1A} receptor increases as the molecular size increases. The presence of the (*R*)-1,2,3,4-tetrahydro[1]benzothieno[2,3-*c*]pyridine skeleton and the attachment of an appropriate hydrophobic group are necessary for the good affinity for the 5-HT_{1A} receptor.

The most active compounds, **71** and **107**, showed anticonflict activity in the water lick conflict test where they were as potent as diazepam. While the compounds increased latency over that seen with diazepam or scopolamine, the ability to reverse scopolamine-induced amnesia in a passive avoidance test was used to assess memory impairment. In particular, the representative compound **107** demonstrated anticonflict activity and lessening of memory impairment with strongly selective 5-HT_{1A} receptor binding.

Table I. Physical Data and 5-HT_{1A} Binding Data of 3-Substituted (*R*)-1,2,3,4-Tetrahydro[1]benzothieno[2,3-c]pyridines



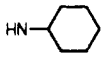
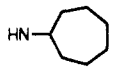
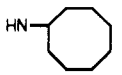
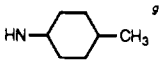
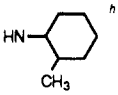
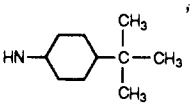
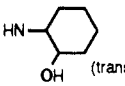
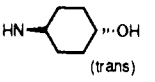
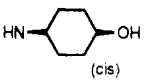
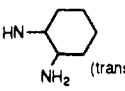
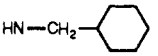
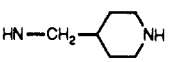
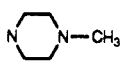
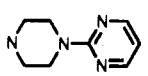
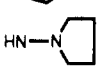
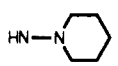
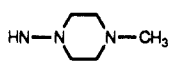
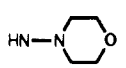
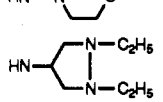
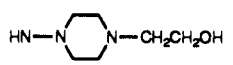
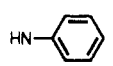
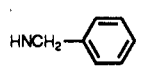
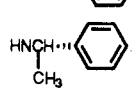
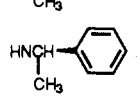
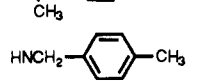
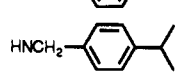
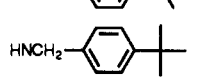
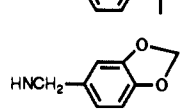
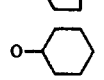
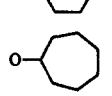
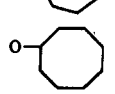
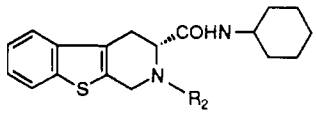
compd	R	yield,[a] %	mp,[b] °C	formula[c]	retention time,[d] min	% inhibition of 5-HT_{1A} binding in compd (10^{-8} M) [IC_{50}[e] (nM) of 5-HT1A]
60		72	243–244	$C_{15}H_{16}N_2OS$·HCl	2.79	10.6
61		74	267–268	$C_{16}H_{18}N_2OS$·HCl	3.56	71.3 [232.4]
62		79	284–285	$C_{17}H_{20}N_2OS$·HCl	4.77	83.0 [110.9]
63		75	264–266	$C_{18}H_{22}N_2OS$·HCl[f]	6.72	92.7 [27.2]
64		79	275–276	$C_{19}H_{24}N_2OS$·HCl	10.56	94.7 [28.4]
65		81	263–264	$C_{20}H_{26}N_2OS$·HCl	15.71	96.0 [22.9]
66		79	268–272	$C_{19}H_{24}N_2OS$·HCl	11.45	94.6 [32.4]
67		82	265–267	$C_{19}H_{24}N_2OS$·HCl	8.99	94.4 [28.0]
68		83	266–268	$C_{22}H_{30}N_2OS$·HCl	68.11	94.5 [52.7]
69		78	278–280	$C_{19}H_{22}N_2OS$·HCl	7.77	89.2 [66.4 ± 5.9]
70		75	277–280	$C_{22}H_{26}N_2OS$·HCl	23.65	91.2 [61.6]
71		62	265–266	$C_{18}H_{22}N_2O_2S$·HCl	3.44	80.6 [148.5 ± 11.0]
72		51	268–270	$C_{18}H_{22}N_2O_2S$·HCl	12.45	32.6
73		49	130–132	$C_{18}H_{22}N_2O_2S$·HCl	2.55	33.7
74		44	298–300	$C_{18}H_{24}N_3OS$·3HCl	2.31	12.4
75		81	252–253	$C_{19}H_{24}N_2OS$·HCl		2.9
76		79	243–245	$C_{19}H_{24}N_2OS$·HCl	12.36	95.5 [20.2]
77		75	282–285	$C_{18}H_{23}N_3OS$·2HCl[k]	5.53	6.3
78	$HN(CH_2)_2CH_3$	77	229–234	$C_{15}H_{18}N_2OS$·HCl	3.35	41.2
79	$HN(CH_2)_3CH_3$	77	248–249	$C_{16}H_{20}N_2OS$·HCl	4.61	63.0
80	$HN(CH_2)_4CH_3$	76	242–243	$C_{17}H_{22}N_2OS$·HCl	7.68	83.2
81	$HN(CH_2)_5CH_3$	72	238–240	$C_{18}H_{24}N_2OS$·HCl	14.40	89.2 [91.2]
82	$HN(CH_2)_6CH_3$	69	236–238	$C_{19}H_{26}N_2OS$·HCl	28.93	84.4 [126.2]
83	$HNCH_2CH(CH_3)_2$	59	271–273	$C_{16}H_{20}N_2OS$·HCl	4.36	64.6
84	$HNC(CH_3)_3$	49	254–259	$C_{16}H_{20}N_2OS$·HCl	4.41	43.1
85	$HNCH_2C(CH_3)_3$	70	256–257	$C_{17}H_{22}N_2OS$·HCl	6.24	81.4
86	$HNCH(CH_2CH_3)_2$	72	249–250	$C_{17}H_{22}N_2OS$·HCl	5.53	66.2 [126.5]
87	$N(CH_2CH_3)_2$	81	254–259	$C_{16}H_{20}N_2OS$·HCl		1.8
88		83	250–252	$C_{17}H_{20}N_2OS$·HCl		0.3

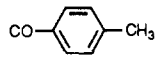
Table I (Continued)

compd	R	yield,[a] %	mp,[b] °C	formula[c]	retention time,[d] min	% inhibition of 5-HT$_{1A}$ binding in compd (10^{-8} M) [IC$_{50}$[e] (nM) of 5-HT1A]
89		72	244–245	$C_{16}H_{18}N_2O_2S$·HCl		0.0
90		31	266–267	$C_{16}H_{19}N_3OS$·2HCl		0.0
91		55	258–260	$C_{17}H_{21}N_3OS$·2HCl		0.1
92		59	252–254	$C_{20}H_{21}N_5OS$·2HCl		2.5
93		35	258–260	$C_{17}H_{21}N_3OS$·2HCl		0.0
94		49	247–248	$C_{16}H_{19}N_3OS$·2HCl[l]	2.31	42.1
95		51	261–263	$C_{17}H_{21}N_3OS$·2HCl	2.88	68.6 [210.9]
96		69	268–270	$C_{17}H_{22}N_4OS$·3HCl		2.9
97		62	261–263	$C_{16}H_{19}N_3O_2S$·2HCl[m]	2.57	21.2
98		60	260–261	$C_{19}H_{26}N_4OS$·3HCl	2.31	73.0
99		68	252–254	$C_{19}H_{24}N_4O_2S$·3HCl		1.9
100		80	268–270	$C_{18}H_{16}N_2OS$·HCl	5.59	68.3 [272.0]
101		79	261–265	$C_{19}H_{18}N_2OS$·HCl	6.03	77.3
102		73	262–266	$C_{20}H_{20}N_2OS$·HCl	8.11	92.5 [58.4]
103		72	245–250	$C_{20}H_{20}N_2OS$·HCl	7.31	79.1
104		71	261–265	$C_{20}H_{20}N_2OS$·HCl	4.36	84.4 [133.1]
105		77	268–274	$C_{22}H_{24}N_2OS$·HCl	29.23	54.4
106		78	285–287	$C_{23}H_{26}N_2OS$·HCl	46.29	24.3
107		81	240–245	$C_{20}H_{18}N_2O_3S$·HCl	5.75	90.0 [53.1 ± 0.7]
108		75	218–221	$C_{17}H_{19}NO_2S$·HCl	10.12	88.1 [120.6]
109		69	286–288	$C_{16}H_{21}NO_2S$·HCl	17.57	93.3 [50.4]
110		71	226–231	$C_{19}H_{23}NO_2S$·HCl	30.13	94.3 [55.2]
111		69	222–224	$C_{20}H_{25}NO_2S$·HCl	52.80	95.0 [67.3]
112	OH	88[n]	>300	$C_{12}H_{11}NO_2S$		1.2
(S)-isomer of 63		71	263–267	$C_{16}H_{22}N_2OS$·HCl		[>5000]
(S)-isomer of 107		79	241–245	$C_{20}H_{18}N_2O_3S$·HCl		[>5000]

[a] The yield comes from G and H synthetic steps in Scheme I. [b] Recrystallization solvent was EtOH/ether. [c] Analyses for C, H, N, S were within ±0.4% of the theoretical values unless otherwise noted. [d] Compound was not shown in inhibition (<5%) of 5-HT$_{1A}$ binding in compd (10^{-8} M) in Table I. HPLC condition: solvent; CH_3CN/0.1 M NH_4Cl = 3:7, solvent speed; 1 mL/min; detected wavelength; 254 nm. [e] Most of the data come from single data determination; however, compounds 69, 71, and 107 were returned in triplicate and are given as their mean ± SEM. [f] S: calcd, 9.14; found, 9.69. [g] These amines were diastereomers. [k] S: calcd, 7.97; found, 8.43. [l] N: calcd, 11.23; found, 11.69. [m] S: calcd, 8.21; found, 7.79. [n] The yield comes from H synthetic step in Scheme I.

Table II. Physical Data and 5-HT_{1A} Binding Data of 2-Substituted (*R*)-1,2,3,4-Tetrahydro[1]benzothieno-[2,3-c]pyridines



compd	R_2	yield,[a] %	mp,[b] °C	formula[c]	% inhibition of 5-HT_{1A} binding in compd (10^{-6} M)
113	CH_3	3.8	260–261	$C_{19}H_{24}N_2OS\cdot HCl$	12.8
114		71	179–181	$C_{20}H_{16}NO_2S$	0.0

[a] The yield comes from I synthetic step in Scheme I. [b] Recrystallization solvent was EtOH/ether. [c] Analyses for C, H, N, S were within ±0.4% of the theoretical values unless otherwise noted.

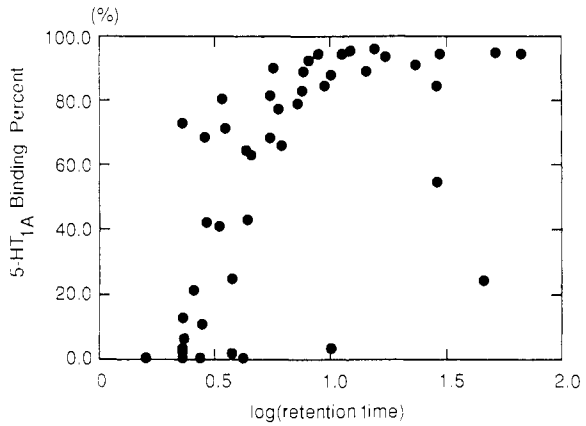


Figure 1. Plot of log (retention time) vs 5-HT_{1A} binding percent.

Table III. Anticonflict Activity on Water Lick Conflict Test in Rats and Effect of Passive Avoidance Test in Scopolamine-Induced Amnesia in Mice of Compounds 71, 107, and Diazepam

compd (mg/kg po)	no. shocks in 5 min (no. of rats)[a,b]	latency to step through, s (no. of mice)[a,c]
control	12.5 ± 2.2 (19)	
diazepam (10)	25.6 ± 2.4*** (43)	
71 (1)	21.6 ± 5.4 (12)	
71 (3)	34.9 ± 7.9** (12)	
71 (30)	26.1 ± 5.5* (7)	
107 (1)	22.1 ± 3.9* (20)	
107 (3)	22.9 ± 4.5* (20)	
107 (10)	28.3 ± 5.5** (14)	
control		279.4 ± 9.2 (18)
scopolamine (1)		16.0 ± 1.6 (18)
diazepam (5)		10.6 ± 6.6*** (15)
71 (3)		68.2 ± 20.0* (18)
71 (10)		41.6 ± 4.7** (18)
71 (30)		50.8 ± 9.2** (18)
107 (1)		94.6 ± 2.3** (18)
107 (3)		89.0 ± 4.5* (18)
107 (30)		104.7 ± 6.9** (18)

[a] *$p < 0.05$; **$p < 0.01$; ***$p < 0.005$ (Student's *t* test). [b] Number of shocks received in 5 min at each dose of compound. [c] Time of latency in seconds at each dose of compound.

Experimental Section

All melting points were determined with a Büchi 510 melting point apparatus and are uncorrected. Elemental analyses performed by Yanagimoto MT-3 were obtained for all new compounds and were within ±0.4% of the theoretical values unless otherwise noted. IR spectra were determined with a Hitachi IR-260-10 spectrometer. NMR spectra were recorded on a NEC JMX GX-400 instrument with tetramethylsilane as an internal standard. MS spectra were measured with an NEC 01-SG mass spectrometer. Column chromatography was carried out with silica gel 60 (0.063–0.200 mm, Wako Pure Chemical Industry, Ltd.). HPLC was carried out with Kaseisorb LC ODS-120-5 (4.5-mm i.d. × 150 mm, Tokyo Chemical Industry Company, Ltd.).

Table IV. Affinity for the 5-HT_{1A} and DA_2 Receptors of Compound 107, Buspirone, and Tandospirone

	compound 107 (M)	buspirone (M)	tandospirone (M)
affinity for the 5-HT_{1A} receptor	$(5.31 \pm 0.07) \times 10^{-8}$	1.5×10^{-8} [a]	2.7×10^{-8} [a]
affinity for the DA_2 receptor	$>1.0 \times 10^{-4}$	9.0×10^{-8} [b]	2.8×10^{-8} [b]

[a] Witkin, J. M.; Mansbach, R. S.; Barrett, J. E.; Bolger, G. T.; Skolnick, P.; Weissman, B. Behavioral Studies with Anxiolytic Drugs. IV. Serotonergic Involvement in the Effects of Buspirone on Punished Behavior of Pigeons. *J. Pharmacol. Exp. Ther. 243*, 970–977. [b] Grattini, S.; Caccia, C.; Mennini, T. Notes on Buspirone's Mechanism of Action. *J. Clin. Psych.* **1943**, *12*, 19–24. Hamik, A.; Oksemberg, D.; Fischette, C.; Peroutka, S. J. Analysis of Tandospirone(SM-3977) Interactions with Neurotransmitter Receptor Binding Sites. *Biol. Psych.* **1990**, *28*, 99–109.

Synthetic Methods. Scheme I. (*R*)-2-Amino-3-[1]benzothiophene-3-ylpropionic Acid (2). Compound 1 (150 g, 570 mmol) was added to H_2O (500 mL), and the pH was adjusted to 6.5 with NaOH. Acylase 3000 units (1.0 g) and $CoCl_2\cdot 6H_2O$ (72 mg, 72 μmol) were added to the reaction mixture, and the resulting mixture was stirred for 1 day at room temperature. Precipitates formed were collected by filtration to provide (*S*)-2-amino-3-([1]benzothiophene-3-yl)propionic acid (59.6 g, 269 mmol), $[\alpha]^{25}_D = -9.17°$ (g/100 mL, 0.1 N HCl). The pH of the reaction solution was adjusted to 6.5 with 6 N HCl. Precipitates formed were collected by filtration to provide (*R*)-α-acetamido-β-[1]benzothiophene-3-ylpropionic acid (73.5 g, 279 mmol). The optical purity was 99.7% ee as determined by HPLC (hexane/2-PrOH/CF_3COOH, 90:10:0.1, Daicel Chemical Industries, Ltd., Chiralcel OJ). (*R*)-α-Acetamido-β-[1]benzothiophene-3-ylpropionic acid (500 g, 1.9 mol) was added to 5 N CH_3SO_3H (5 L), and the mixture was stirred for 7 h at 100 °C. After cooling, H_2O was added, and the pH was adjusted to 6.5 with 6 N NaOH. Precipitates formed were collected by filtration to provide (*R*)-2-amino-3-[1]benzothiophene-3-ylpropionic acid (400 g, 1.52 mol), $[\alpha]^{25}_D = 9.80°$ (g/100 mL, 0.1 N HCl). IR (KBr): 3050, 1670, 1585 cm^{-1}.

(*R*)-α-Amino-3-[1]benzothiophene-3-ylpropionic Acid Methyl Ester Hydrochloride (3). Thionyl chloride (260 mL, 1.32 mol) was gradually added to dried MeOH (1.5 L) at −10 °C. The mixture was stirred for 30 min at −10 °C. Compound 2 (221.3 g, 1.00 mol) was added to the mixture, and the resulting mixture was stirred for 2 days at room temperature. After MeOH was removed under reduced pressure, Et_2O (500 mL) was added to the residue. Compound 3 (323.9 g, 856 mmol) was obtained by filtration in a yield of 90%. Mp: 156–157 °C. IR (KBr): 1740 cm^{-1}. Anal. ($C_{12}H_{14}NO_2SCl$): C, H, N, S.

(*R*)-1,2,3,4-Tetrahydro[1]benzothieno[2,3-*c*]pyridine-3-carboxylic Acid Methyl Ester (4). Compound 3 (110.6 g, 407 mmol) and 30% HCHO (51 mL, 510 mmol) were dissolved in MeOH (1 L) and H_2O (1 L), and the mixture was stirred at 80 °C for 5 h. The reaction mixture was concentrated to about half its original volume. The pH was adjusted to 10 with $NaHCO_3$. The mixture was extracted with $CHCl_3$ (100 mL × 3). The $CHCl_3$ layer was washed with saturated aqueous brine (50 mL × 2) and dried on Na_2SO_4. $CHCl_3$ was removed under reduced pressure. The residue was recrystallized from $CHCl_3$ (100 mL) and Et_2O (100 mL) to provide compound 4 (74.1 g, 296 mmol) in a yield of 72%. Mp: 128–130 °C. IR (KBr): 2970, 2900, 1740, 1440, 1160, 760, 740 cm^{-1}. NMR (δ, $CDCl_3$): 2.25 (s, 2H), 3.10 (d, J = 2.5 Hz, 2 H), 3.72–4.00 (m, 1 H), 4.23 (s, 3 H), 7.01–8.02 (m, 4 H). Anal. ($C_{13}H_{13}NO_2S$): C, H, N, S.

2-(*tert*-Butoxycarbonyl)-(*R*)-1,2,3,4-tetrahydro[1]-benzothieno[2,3-*c*]pyridine-3-carboxylic Acid Methyl Ester (5). Compound 4 (23.9 g, 96.6 mmol) and $(Boc)_2O$ (23.9 g, 96.6 mmol) were dissolved in dry THF (30 mL), MeOH (80 mL), and NEt_3 (16.2 mL, 116 mmol), and the mixture was stirred at 60 °C for 2 h. The THF and MeOH solution was concentrated under

reduced pressure. The residue was dissolved in AcOEt (300 mL), and the solution was washed with an aqueous 10% citric acid solution (50 mL × 2), an aqueous 5% $NaHCO_3$ solution (50 mL × 2), and saturated aqueous brine (50 mL × 2) and dried on Na_2SO_4. AcOEt was removed under reduced pressure, and compound **5** (32.6 g, 93.7 mmol) was recrystallized from $CHCl_3$ (60 mL) and petroleum ether (30 mL) in a yield of 79%. Mp: 179–181 °C. IR (KBr): 1740, 1700 cm^{-1}. Anal. ($C_{18}H_{21}NO_4S$): C, H, N, S.

2-(*tert*-Butoxycarbonyl)-(*R*)-1,2,3,4-tetrahydro[1]benzothieno[2,3-*c*]pyridine-3-carboxylic Acid (6). Compound **5** (5.77 g, 17.4 mmol) and 5 N NaOH (3.5 mL, 17.6 mmol) were dissolved in MeOH (50 mL), stirred at 100 °C for 1 h, and concentrated under reduced pressure. Aqueous 5% citric acid (300 mL) and $CHCl_3$ (300 mL) were added to the residue, and the mixture was shaken. The $CHCl_3$ extract was dried on Na_2SO_4 and concentrated to give **6** (91%) and **7** (9%) (5.03 g, 15.8 mmol). The mixture was dissolved in *i*-PrOH (50 mL) and added to D-(+)-1-phenyethylamine (1.91 g, 15.8 mmol), and then the mixture was stirred at 25 °C for 1 h and recrystallized in MeOH (25 mL), giving 2-(*tert*-butoxycarbonyl)-(*R*)-1,2,3,4-tetrahydro[1]benzothieno[2,3-*c*]pyridine-3-carboxylic acid D-(+)-1-phenyethylamine salt (5.97 g, 13.6 mmol). This compound (5.97 g, 13.6 mmol) and 1 N HCl (31 mL) were dissolved in CH_2Cl_2 (30 mL) and stirred at 20 °C for 1 h, and the solution was washed with an aqueous 10% citric acid solution (50 mL × 2) and saturated aqueous brine (50 mL × 2), dried on Na_2SO_4, and then removed under reduced pressure. Compound **6** (4.41 g, 13.3 mmol) was recrystallized from AcOEt (10 mL) and *n*-hexane (30 mL) in a yield of 76%. Mp: 212–214 °C. IR (KBr): 1700, 1695 cm^{-1}. Anal. ($C_{17}H_{19}NO_4S$): C, H, N, S.

2-(*tert*-Butoxycarbonyl)-(*R*)-*N*-cyclopropyl-1,2,3,4-tetrahydro[1]benzothieno[2,3-*c*]pyridine-3-carboxamide (8). Compound **6** (3.3 g, 10 mmol) and cyclopropylamine (0.63 g, 11 mmol) were dissolved in DMF (20 mL). A solution of DPPA (3.3 g, 12 mmol) in DMF (10 mL) and a solution of NEt_3 (1.53 mL, 11 mmol) in DMF (5 mL) were added to the solution under cooling and stirred at 0 °C for 1 h and further at 20 °C for 12 h. AcOEt (100 mL) was added to the reaction mixture. The mixture was washed with aqueous 5% citric acid (20 mL × 2), aqueous Na_2CO_3 (20 mL × 2), and saturated aqueous brine (20 mL × 2). The AcOEt extract was dried on Na_2SO_4, concentrated, and purified through a silica gel column ($CHCl_3$) to give **8** (2.88 g, 7.7 mmol) in a yield of 77%. Mp: 182–184 °C. IR (KBr): 1700, 1670 cm^{-1}. Anal. ($C_{20}H_{24}N_2O_3S$): C, H, N, S.

(*R*)-*N*-Cyclopropyl-1,2,3,4-tetrahydro[1]benzothieno[2,3-*c*]pyridine-3-carboxamide Hydrochloride (60). Compound **8** (2.88 g, 7.7 mmol) and 1 N HCl in AcOEt solution (10 mL) were dissolved in AcOEt (20 mL) at 80 °C for 1 h. The reaction mixture was allowed to stand overnight in a refrigerator, and the precipitated crystals were collected to give **60** (2.12 g, 6.86 mmol) in a yield of 89%. The optical purity was 99.8% ee as determined by HPLC (hexane/EtOH, 6:4, Daicel Chemical Industries, Ltd., Chiralpak AS). Mp: 243–244 °C. IR KBr): 3250, 2940, 2700, 2600, 1690, 1680, 1540, 765, 740 cm^{-1}. NMR (δ, $CDCl_3$): 0.50–0.83 (m, 4 H), 2.81–3.24 (m, 1 H), 3.45–3.89 (m, 2 H), 4.16–4.50 (m, 1 H), 4.93–5.32 (m, 2 H), 5.72–5.98 (m, 1 H), 7.12–7.48 (m, 2H), 7.65–7.82 (m, 2 H). Anal. ($C_{15}H_{16}N_2OS$·HCl): C, H, N, S.

(*R*)-1,2,3,4-Tetrahydro[1]benzothieno[2,3-*c*]pyridine-3-carboxylic Acid (112). Compound **6** (3.17 g, 10 mmol) and 1 N HCl in AcOEt solution (12 mL) were dissolved in AcOEt (20 mL) at 80 °C for 1 h. The reaction mixture was allowed to stand overnight in a refrigerator, and the precipitated crystals were collected to give **112** (2.05 g, 8.88 mmol) in a yield of 88%. The optical purity was 99.9% ee as determined by HPLC (25 mM $CuSO_4$, Daicel Chemical Industries, Ltd., Chiralcel WH.). Mp: >300 °C. IR (KBr): 3250, 2940, 2700, 2600, 1690, 1680, 1540, 765, 740 cm^{-1}. NMR (δ, DMSO-d_6): 2.38–4.44 (m, 5 H), 6.20–7.48 (m, 4 H). Anal. ($C_{12}H_{11}NO_2S$): C, H, N, S.

(*R*)-*N*-Cyclohexyl-2-methyl-1,2,3,4-tetrahydro[1]benzothieno[2,3-*c*]pyridine-3-carboxamide Hydrochloride (113). Compound **63** (10.5 g, 30 mmol), MeI (1.86 mL, 30 mmol), and NEt_3 (6.7 g, 66 mmol) were dissolved in $CHCl_3$ (100 mL) and stirred at room temperature for 18 h. The reaction mixture was diluted with $CHCl_3$ (50 mL) and washed with aqueous $NaHCO_3$ (30 mL) and with H_2O (30 mL × 2). The $CHCl_3$ solution was dried on Na_2SO_4, concentrated, and purified through a silica gel column ($CHCl_3$/MeOH, 50:1). The oil product was dissolved in MeOH (50 mL), and a MeOH solution containing 1 N HCl (30 mL) was added to the residue. Precipitated crystals were collected by filtration to provide **113** (7.15 g, 19.6 mmol) in a yield of 65%. The optical purity was 99.9% ee as determined by HPLC (hexane/EtOH, 60:35, Daicel Chemical Industries, Ltd., Chiralpak AS). Mp: 260–261 °C. IR (KBr): 3220, 2940, 2860, 1680, 1545, 760, 730 cm^{-1}. NMR (δ, DMSO-d_6): 0.73–2.16 (m, 10 H), 2.56 (m, 3 H), 2.93–3.30 (m, 1 H), 3.40–3.90 (m, 2 H), 3.93–4.50 (m, 1 H), 4.83–4.98 (m, 2 H), 5.67–5.85 (m, 1 H), 7.16–7.90 (m, 4 H). Anal. ($C_{18}H_{24}N_2OS$·HCl): C, H, N, S.

5-HT$_{1A}$ Receptor Binding Assays.[16] Tissue preparation: Male Sprague–Dawley rats (200–250 g, Charles River) were sacrificed by decapitation and their brains removed rapidly and placed in 0.32 M sucrose on ice. The hippocampus was dissected and homogenized in 10 volumes of 50 mM Tris-HCl buffer (pH 7.4). The homogenate was centrifuged at 48000*g* for 15 min. The resulting pellet was resuspended in Tris buffer and incubated at 30 °C for 20 min followed by centrifugation at 48000*g* for 15 min. The final pellet was suspended in the binding buffer (50 mM Tris pH 7.7, 0.1% ascorbic acid, 4 mM $CaCl_2$, and 10 μM pargyline). [^{3}H-5-HT binding studies: Tissue preparations (150-200 μg protein/tube) were incubated in duplicate with 0.5 nM [^{3}H]-8-OH-DPAT (5901.5 GBq/mmol, NEN) in the presence of unlabeled drugs for 30 min at 30 °C. Nonspecific binding was determined with 10 μM 5-HT. The binding reaction was terminated by filtration under vacuum through GF/C filters. Filters were washed twice with 5 mL of the binding buffer. Radioactivity was determined using an aliquot scintillation counter. The K_i values were calculated from the IC_{50} by the formula: $K_i = IC_{50}/(1 + L^*/K^*)$ where K^* is the dissociation constant of the labeled ligand and L^* is the concentration of labeled ligand.

Water Lick Conflict Test.[17] Male Wistar rats (170–200 g) were used. Rats were deprived of water for 24 h prior to the test. The rats were placed in a test chamber and allowed to locate a drinking spout which gave off mild electric shocks (1.0 mA). Only rats making between 3 and 10 licks during a total of 30 s in this presession were used for the animal test. The rats were removed from the chamber, administered compounds and were returned to the home cage for 60 min. The rats were then returned to the test chamber for the 5-min test session. During the 5-min test session, the rats were delivered a shock (2.5 mA) for every drop they drank. The number of shocks received during the 5 min session was recorded automatically.

Passive Avoidance Test.[18,19] Male ddy mice (20–25 g) were kept in a plastic cage in groups of 10 and allowed free access to dry food pellets and water. The mice were trained in a one-trial step-through passive avoidance task. The apparatus consisted of a small white box (15 cm × 10 cm × 9 cm) and a black shock box (15 cm × 15 cm × 14 cm) with a hall at the bottom and a grillotine door. During the acquisition test, they were placed in a small lighted compartment. Five seconds later, the door was opened. The mice received a 1 mA foot shock after entering the dark compartment and they were then returned to their home cage. Twenty-four hours later, after the acquisition test, mice were placed in the small lighted box again. Time latency to enter the dark compartment was recorded (maximum = 300 s). Compounds were administered 60 min prior to the acquisition. For evaluation of the effect on scopolamine-induced amnesia, scopolamine 3 mg/kg was administered intraperitoneally 15 min prior to the acquisition test. Compounds were administered 45 min prior to the administration of scopolamine.

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