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New Thiazole Derivatives as Potent and Selective 5-Hydroxytriptamine 3 (5-HT_3) Receptor Agonists for the Treatment of Constipation

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Abstract—The syntheses and biological evaluation of a series of novel indeno[1,2-*d*]thiazole derivatives are described. Several groups reported 5-HT_3 receptor agonists which were mainly evaluated for their activities on the von Bezold–Jarisch reflex (B–J reflex). We discovered that tetrahydrothiazolopyridine derivative **1b** had a contractile effect on the isolated guinea pig colon with weak B–J reflex. Our efforts to find a new type of 5-HT_3 receptor agonists on the isolated guinea pig colon focused on the synthesis of a fused thiazole derivative **1d** modified from **1b** and reverse-fused thiazole derivatives (**7**–**10**). In this series, **10f** (YM-31636) showed high affinity and selectivity for the cloned human 5-HT_3 receptor; furthermore, it showed potent and selective 5-HT_3 receptor agonistic activity. YM-31636 was examined for its effects on defecation in animals, thus evaluating the compound as an agent against constipation.

Introduction

Constipation is one of the most common chronic digestive disorders. The definition of constipation, however, is not sufficiently established, probably because of its various kinds of symptoms. The principal cause of the development of constipation is the motility disorder of the colon; functional constipation is classified into two main categories, that is, atonic and spastic constipation.[1] Laxatives are widely used in the treatment of constipation, although some of them are contraindicated in spastic constipation. Their onset of action is slow and dispersed, causing difficulty in control of the time of defecation. Moreover, their main action is to increase water secretion from the colonic mucosa. They, therefore, tend to cause diarrhea, dehydration and electrolyte disturbance.

5-Hydroxytryptamine (5-HT) is a biogenic amine that mediates a variety of physiological actions. In the gastrointestinal tract, 5-HT is stored in and released from enterochromaffin (EC) cells and enteric serotonergic neurons.[2a,b] A 5-HT_3 receptor was identified on enteric neurons.[3a,b] Selective 5-HT_3 receptor antagonists suppress stress- or 5-HT-induced defecation in rats[4] and reduce 5-HT-induced colonic motility in rats and dogs.[5a,b] It has also been reported that 5-HT_3 receptor antagonists inhibit colonic motility in healthy humans,[6] and decrease defecation frequency in women with irritable bowel syndrome.[7] The mechanism of the regulation of colonic function through the 5-HT_3 receptor is considered to be as follows; 5-HT is released in compliance with stimulation of mucosal surface by intralurminal contents, and then binds to the neuronally located 5-HT_3 receptors. As a result, colonic functions are accelerated through the release of acetylcholine, tachykinins, VIP and/or NO. 5-HT_3 receptor stimulating agents, therefore, are expected to be new promising candidate for the treatment of constipation.

Several groups have reported 5-HT_3 receptor agonists[8–16] identified with their activity on the 5-HT-evoked reflex bradycardia (von Bezold–Jarisch reflex; B–J reflex).[17] We previously reported that thiazole derivatives, for example

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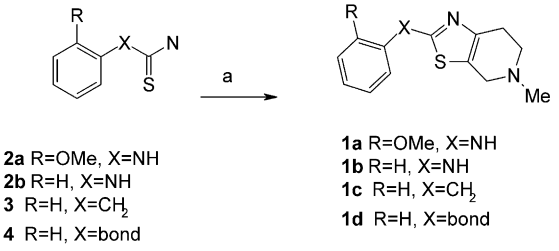
2 - (*o* - methoxyanilino) - 5 - methyl - 4,5,6,7 - tetrahydrothiazolo[5,4-*c*] pyridine **1a**,[18] as a 5-HT_3 receptor agonist which stimulated the B–J reflex. But the B–J reflex is an undesirable side effect against the treatment of constipation.

We have paid attention to the contractile effects of 5-HT through the 5-HT_3 receptor in the gastrointestinal tract. We have discovered that compound **1b**, the demethoxy derivative of **1a**, had a contractile effect on the isolated guinea pig colon as an index for the 5-HT_3 receptor agonistic activity, independent of the B–J reflex.

Our efforts to find a new type of 5-HT_3 receptor agonists on the isolated guinea pig colon focused on the synthesis of a fused thiazole derivative **1d** modified from **1b** and reverse-fused thiazole derivatives **7–10** in which the fused ring is reversed to an aromatic ring from the aliphatic amine part of **1d** (Fig. 1).

Chemistry

A series of tetrahydrothiazolopyidines were prepared from thiourea or thiamide by condensation with haloketones in 2-propanol in good yield (Scheme 1).



Scheme 1. Syntheses of 5,6,7,8-tetrahydrothiazolopyridine derivatives: (a) 3-bromo-1-methyl-4-piperidone hydrobromide, iPrOH.

A series of indenothiazoles were prepared from 2-bromo-1-indanone by condensation with thioamides or thioureas in 2-propanol. The primary amine containing compounds were generated from phthalimido derivatives (Scheme 2).

Results and Discussion

The synthesized compounds were assessed for 5-HT_3 agonistic activities with their contractile effect on the isolated guinea pig colon[19] and potent compounds were assessed for the B–J reflex in rat as an index for a side effect against the cardiovascular system.[20] 5-HT caused a dose-dependent contraction within its concentration range of 0.1–30 μM and showed the maximum response at 10–30 μM; the action of 5-HT is mediated via the 5-HT_3 receptor. Activity of each compound is expressed by a relative value in comparison with the activity of 5-HT in each specimen. The intrinsic activity (relative efficacy compared to 5-HT) is indicated as a percentage of the maximum response by each compound when the maximum contraction by 5-HT is defined as 100%. The relative potency compared to 5-HT is determined according to the following equation; Relative potency = EC_{50} value for the 5-HT/EC_{50} value for a compound. The contractile effect of each compound was antagonized by 0.3 μM ramosetron which is a selective 5-HT_3 receptor antagonist. Further, the potent compounds were assessed in a [^{3}H]-ramosetron binding study on the cloned rat 5-HT_3 receptor.[21]

Compound **1a** was a potent 5-HT_3 agonist as the B–J reflex inducer. Demethoxy compound **1b** reduced 5-HT_3 agonistic activity, but increased the contractile effect on the isolated guinea pig colon. Replacement of the linker nitrogen atom of **1b** by a methylene group lost the 5-HT_3 agonistic activity. Compound **1d,** with the benzene ring that was directly connected to the thiazole

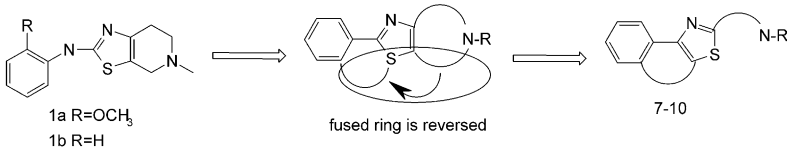
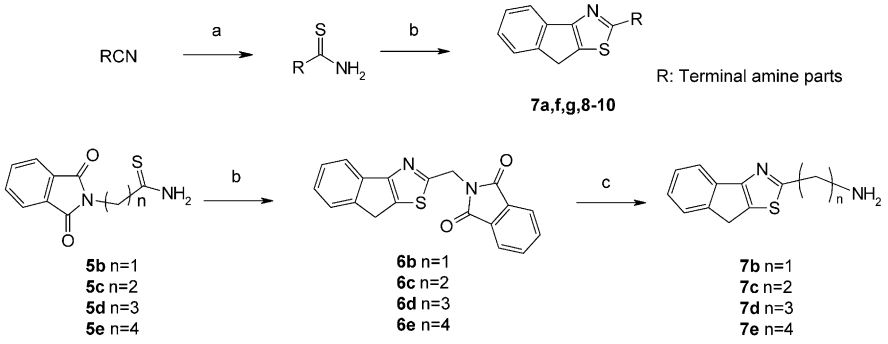
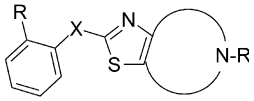


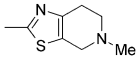
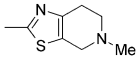
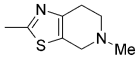
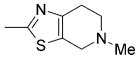
Figure 1. Synthetic plan.



Scheme 2. Synthesis of 2-substituted indenothiazoles: (a) $(EtO)_2P(:S)SH$, 4 N HCl in EtOAc; (b) 2-bromo-1-indanone, iPrOH; (c) $MeNH_2$, MeOH.

Table 1. 5-HT_3 receptor agonistic activities and binding profile of the fused-thiazole derivatives



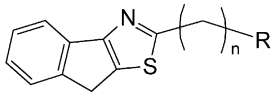
No.	R	X	Fused thiazole moieties	Guinea pig colon contraction		Rat B–J reflex		5-HT_3 binding[e]
				Intrinsic activity (%)[a]	Relative potency (fold)[b]	Maximum response (%)[c]	Relative potency (fold)[d]	IC_{50}(nM)[f]
1a	OCH_3	NH		8	—	80	3	14.4
1b	H	NH		57	0.3	55	0.25	390
1c	H	CH_2		0	—	NT	NT	>1000
1d	H	bond		58	1	26	<0.33	113
5-HT				100[g]	1	100[h]	1	150

—, not determined; NT, not tested.
[a]The intrinsic activity (relative efficacy compared to 5-HT) was indicated as a percentage of the maximum response by each compound when the maximum contraction by 5-HT is defined as 100%.
[b]The relative potency compared to 5-HT was determined according to the following equation. relative potency = EC_{50} value for the 5-HT/EC_{50} value for a compound.
[c]Maximum response was indicated by the percentage of the maximum response by each compound when the maximum response by 5-HT was defined as 100%.
[d]The relative potency compared to 5-HT was determined according to the following equation. relative potency = ED_{50} value for the 5-HT/ED_{50} value for a compound.
[e][^{3}H]-ramosetron was used as a 5-HT_3 receptor ligand.
[f]IC_{50} values determined by duplicate ($n = 1$).
[g]The EC_{50} value for 5-HT was 3.1 μM.[19]
[h]The ED_{50} value for 5-HT was 15.5 μg/kg iv.

moiety, had potent 5-HT_3 agonistic activity for colonic contraction in spite of weak B–J reflex activity (Table 1).

The next step, indenothiazoles, in which a fused ring as reversed to an aromatic ring from the aliphatic amine part of **1**, were assessed for contractile effect. The distance between the indenothiazole ring and amino group was varied; C2–C3 methylene length was favorable as a linker group (Table 2).

Table 2. 5-HT_3 receptor agonistic activities and binding profile of the 2-(aminoalkyl)indenothiazoles



No.	*n*	R	Guinea pig colon contraction		5-HT_3 binding[e]
			Intrinsic activity (%)[a]	Relative potency (fold)[b]	IC_{50} (nM)[f]
7a	0	NH_2	0	—	>1000
7b	1	NH_2	0	—	>1000
7c	2	NH_2	75	0.5	8.2
7d	3	NH_2	52	1	4.3
7e	4	NH_2	0	—	3.5
7f	2	NMe_2	76	3	1.2
7g	3	NMe_2	47	10	1.4

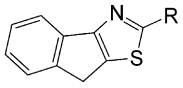
—, not determined; NT, not tested.
[a,b,e,f]Refer to Table 1.

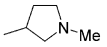
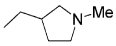
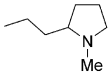
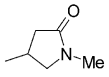
Further, the chain length between the aromatic ring and the nitrogen atom were fixed at 2 or 3 carbon lengths and the terminal amino groups were changed into cyclic amines. Among the cyclic aliphatic amines, 3-pyrrolidine derivatives **8a** and **8f** showed similar potent contractile activity and also stimulated the B–J reflex, and they were not enough separation. Compound **8e** showed partial agonistic activity in colonic contraction without inducing B–J reflex. Lactam compound **9** had no activity, so it was found that the basic nitrogen atom was necessary for the activity (Table 3).

Next, aromatic amine derivatives like pyridines, imidazoles were assessed in Table 4. Pyridine ring containing compound **10a** showed weak activity. Changing the substitution position was not effective with the pyridine derivatives. However, compounds containing an imidazole ring showed potent colon contractile activities. Their activities were marginally affected by the substitution position. Especially, 4-substituted compound **10f** (YM-31636) showed almost full agonistic activities in inducing contraction with an intrinsic activity of 0.90- and 26-fold potency compared to 5-HT in isolated guinea pig distal colon (Fig. 2). As regarding the chain length, arylmethyl ($n = 1$) was more favorable than an ethyl group (**10f** and **10i**).

We selected YM-31636 for further confirmation to characterize as a 5-HT_3 receptor agonist in other assays.

Table 3. 5-HT_3 receptor agonistic activities and binding profile of the aliphatic amine containing indenothiazoles



No.	R	Guinea pig colon contraction		Rat B–J reflex		5-HT_3 binding[a]
		Intrinsic activity (%)[a]	Relative potency (fold)[b]	Maximum response (%)[c]	Relative potency (fold)[d]	IC_{50} (nM)[a]
8a		75	14	97	4.2	1.54
8b		79	3	30	—	7.17
8c		76	4	NT	NT	12.3
8d		78	6	NT	NT	NT
8e		26	4	0.4 @1 mg/kg	—	NT
8f		55	10	75	12	NT
9		2.4	—	NT	NT	NT

—, not determined; NT, not tested.
[a–f]Refer to Table 1.

Until recently, only one subunit of the 5-HT_3 receptor had been cloned, but a distinct subunit, 5-HT_{3B}, has been discovered.[22] Transcripts of this subunit are co-expressed with the established 5-HT_3 receptor subunit, 5-HT_{3A}, in a specific brain region. YM-31636 showed extremely higher affinity for the cloned human 5-HT_{3A} receptor compared to the other compounds known as 5-HT_3 agonist (Table 5). Additionally, YM-31636 did not show affinities to other receptors as shown in Table 6.

In these results, YM-31636 showed the most potent 5-HT_3 receptor binding affinity and colon selectivity in vitro tests.

We examined the anticonstipation effect of YM-31636 on defecation in ferrets.[23] YM-31636 facilitated defecation within 1 h, mostly within 30 min, after oral administration in ferrets (Fig. 3). As shown in Figure 4, YM-31636 significantly increased the frequency of

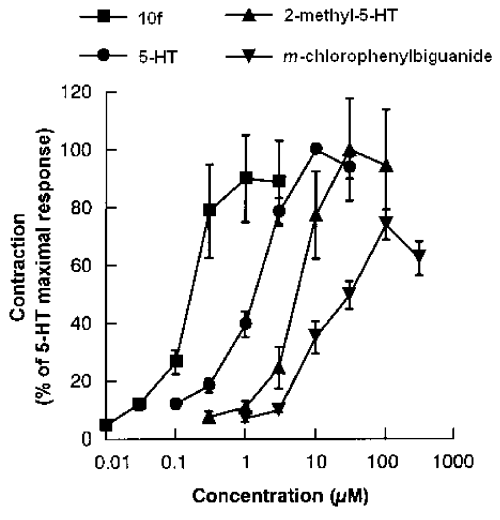


Figure 2. The effects of **10f**, 5-HT, 2-methyl-5-HT and *m*-chlorophenylbiguanide ($n=6$) on contraction of isolated guinea pig distal colon. Segments of the distal colon approximately 20 mm long were suspended longitudinally in an organ bath containing Krebs-bicarbonate solution; isometric contractions were recorded. Concentration–response curves were constracted in a cumulative manner. Each point represents the mean ± SEM.

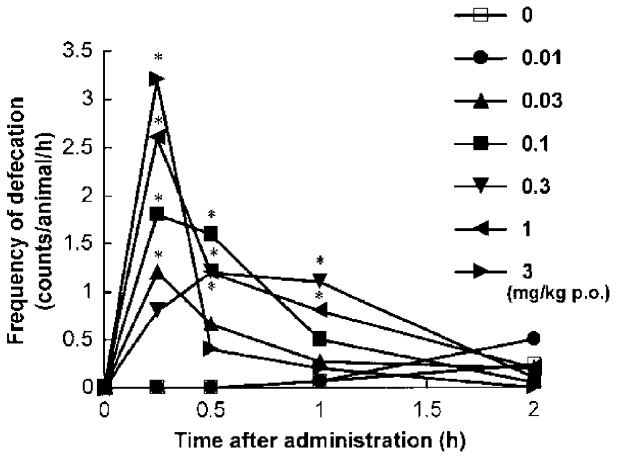
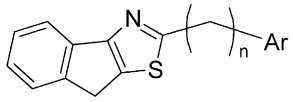


Figure 3. **10f** facilitated defecation after oral administration in ferrets. The between-subjects and within-subjects effects and the interaction were significantly different by two-way repeated measures ANOVA.

Table 4. 5-HT_3 receptor agonistic activities and binding profile of the aromatic amine containing indenothiazoles



No.	*n*	Ar	Guinea pig colon contraction		Rat B–J reflex		5-HT_3 binding[a]
			Intrinsic activity (%)[a]	Relative potency (fold)[b]	Maximum response (%)[c]	Relative potency (fold)[d]	IC_{50}(nM)[a]
10a	1		13	1/6	NT	NT	185
10b	1		0	—	NT	NT	41.6
10c	1		0	—	NT	NT	83.6
10d	1		0	—	NT	NT	NT
10e	1		61	3	NT	NT	11.7
10f	1		90	26	28 @1 mg/kg	<0.01	0.40
10g	2		0	—	NT	NT	457
10h	2		99	4	NT	NT	12.8
10i	2		64	12	NT	NT	0.70

—, not determined; NT, not tested.
[a–f]Refer to Table 1.

defecation within 1 h from 0.03 mg/kg po administration and this effect was inhibited by ramosetron, a selective 5-HT_3 receptor antagonist. These results suggest that YM-31636 is expected to be a new type of agent against constipation which has high potency and efficacy. The results also indicate that YM-31636 has an early and restricted onset of action compared to existing laxatives.

In conclusion, we found that YM-31636 (**10f**) was a potent and selective 5-HT_3 receptor agonist. YM-31636 facilitated defecation with high potency and efficacy. YM-31636 would be promising in the treatment of constipation of both atonic and spastic types, such as atonic constipation, irritable bowel syndrome (constipation-predominant), morphine-induced constipation, post-operative ileus, symptomatic constipation (diabetes mellitus, Parkinson's disease, etc.), chronic colonic obstruction and colonic inertia.

Table 5. Affinity of 5-HT_3 receptor agonists for cloned human 5-HT_{3A} receptors[21]

Compd	pK_i (nM)	*n*H
5-HT	6.47±0.17	0.81±0.07
2-Methyl-5-HT	6.18±0.09	1.10±0.13
m-Chlorophenylbiguanide	6.65±0.07	0.91±0.09
YM-31636	9.67±0.05	0.89±0.19

Each value represents mean±SEM ($n=3$). Radioligand used was [^{3}H]ramosetron.

Table 6. Affinity of YM-31636 for other receptors[21]

Receptor	[^{3}H] ligand	Tissue	K_i (nM)
α_1-Adrenergic	Prazosin	Rat brain	>10,000
α_2-Adrenergic	Rauwolsine	Rat brain	1300±80
β-Adrenergic	DHA	Rat brain	>10,000
Benzodiazepine	Flunitrazepam	Rat brain	>10,000
Dopamine D_1	SCH23390	Rat striatum	>10,000
Dopamine D_2	Racropride	Rat striatum	>10,000
Histamine H_1	Pyrilamine	g.p. brain	>10,000
Muscarinic	QNB	Rat brain	>10,000
Opiate	Naloxone	Rat brain	>10,000
5-HT_1	5-HT	Rat brain	>10,000
5-HT_2	Ketanserin	Rat brain	>10,000
5-HT_3	GR65630	Rabbit illeum	0.20±0.02

Each value represents mean±SEM ($n=3$).

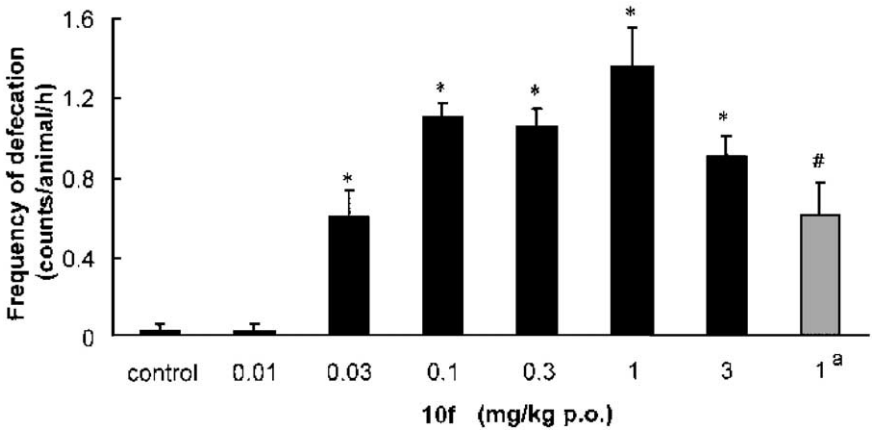


Figure 4. The effect of **10f** on defecation in normal ferrets. **10f** (0.01–3 mg/kg, $n = 10$–30) was administered orally and the frequency of defecation was observed. Each value represents the mean ± SEM for 1 h after administration. (a) Pretreatment with ramosetron, 1 μg/kg sc, 0.5 h before administration of YM-31636. $^{*}p < 0.05$ compared with control group with Dunnett's test. $^{\#}p < 0.05$ compared with YM-31636 (1 mg/kg) treatment group with Student's *t*-test.

Experimental

Chemistry

All melting points were determined on a Yanaco MP-500D melting point apparatus without correction. ^{1}H NMR spectra were measured with a JEOL FX90Q, a FX100, a FX270 or FX400 spectrometer; chemical shifts are reported in δ units using tetramethylsilane as internal standard and the following abbreviations are used: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = double doublet, dt = double triplet, br = broad. Mass spectra were recorded with a Hitachi M-80 electron impact (EI), JEOL JMS-DX300 (FAB) spectrometer or Hewlett Packard 5970 MSD (GC) spectrometer. Elemental analyses were performed with a Yanaco MT-5. All organic extracts were dried over anhydrous magnesium sulfate and concentrated with a rotary evaporator under reduced pressure.

2-Anilino-5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridine (1b). A solution of phenylthiourea **2b** (0.30 g, 1.97 mmol) and 3-bromo-1-methylpiperidin-4-one hydrobromide (0.54 g 1.98 mmol) in 2-propanol (5 mL) solution was stirred under reflux for 1 h and concentrated in vacuo. The residue was dissolved in ethyl acetate, which was washed with saturated aqueous $NaHCO_3$, H_2O, brine and dried over $MgSO_4$, then concentrated in vacuo to afford the crude aminothiazole. This oil was dissolved in ethanol and treated with fumaric acid. **1b** was obtained as a hemifumarate (0.12 g 17% yield): ^{1}H NMR (DMSO-d_6) δ 2.48 (3H, s), 2.65 (2H, br), 2.85 (2H, br), 3.60 (2H, s), 6.62 (1H, s), 6.91 (1H, t, $J = 7.3$ Hz), 7.28 (2H, t, $J = 7.9$ Hz), 7.59 (2H, d, $J = 8.0$ Hz), 10.04 (1H, br); EI-MS *m/e* (M^+) 245; Anal. calcd for $C_{13}H_{15}N_3S{\cdot}0.5C_4H_4O_4{\cdot}0.5C_2H_5OH{\cdot}H_2O$: C, 55.80; H, 6.44; N, 12.20; S, 9.31. Found: C, 55.71; H, 6.05; N, 11.89; S, 9.52; mp 214–216 °C.

5-Methyl-2-phenyl-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridine (1d). A solution of thiobenzamide **4** (1.40 g, 10.2 mmol), 3-bromo-1-methylpiperidin-4-one hydrobromide (3.00 g 11.0 mmol), and calcium carbonate (6.00 g) in 2-propanol (30 mL) solution was stirred under reflux for 10 h and concentrated in vacuo. The residue was dissolved in ethyl acetate, and the organic layer was extracted with aqueous 1 N HCl. The organic layer was separated and discarded. The aqueous layer was basified with aqueous saturated $NaHCO_3$ and extracted with $CHCl_3$. The organic layer was washed with H_2O, brine and dried over $MgSO_4$, then concentrated in vacuo. The residue was purified by column chromatography on silica gel eluting with 50:1:0.1 $CHCl_3$/methanol/29% aqueous ammonia. The obtained free amine was dissolved in methanol and treated with fumaric acid. **1d** was obtained as a fumarate (0.24 g 6.8% yield): ^{1}H NMR (DMSO-d_6) δ 2.42 (3H, s), 2.77–2.85 (4H, m), 3.68 (2H, s), 6.62 (2H, s), 7.45–7.50 (3H, m), 7.86–7.88 (2H, m); EI-MS *m/e* (M^+) 230; Anal. calcd for $C_{13}H_{14}N_2S{\cdot}C_4H_4O_4{\cdot}0.5C_2H_5OH{\cdot}0.2H_2O$: C, 58.34; H, 5.30; N, 8.00; S, 9.16. Found: C, 58.18; H, 5.23; N, 7.84; S, 9.26; mp 185–188 °C.

2-Benzyl-5-methyl-4,5,6,7-tetrahydrothiazolo[5,4-*c*]pyridine (1c). Compound **1c** was synthesized from phenylthioacetoamide **3** and 3-bromo-1-methylpiperidin-4-one hydrobromide according to the same procedure as that for **1d**. Compound **1c** was obtained as a fumarate (yield 9.3%): ^{1}H NMR (DMSO-d_6) δ 2.38 (3H, s), 2.74 (4H, s), 3.57 (2H, s), 4.24 (2H, s), 6.61 (2H, s), 7.27–7.37 (5H, m); EI-MS *m/e* (M^+) 244; Anal. calcd for $C_{14}H_{16}N_2S{\cdot}C_4H_4O_4$: C, 59.98; H, 5.59; N, 7.77; S, 8.90. Found: C, 59.74; H, 5.63; N, 7.65; S, 8.82; mp 160–161 °C.

Phthalimido-thioacetamide (5b). A solution of aminoacetonitrile hydrochloride (2.30 g 24.9 mmol), triethylamine (3.46 mL, 24.9 mmol), and phthalic anhydride (3.70 g, 24.9 mmol) in $CHCl_3$ (30 mL) was stirred under reflux for 6 h. The reaction mixture was poured into H_2O; then the organic layer was separated. The organic layer was washed with H_2O, brine and dried over $MgSO_4$, and then concentrated in vacuo to afford phthalimideacetonitrile (3.07 g, quantitative). The acetonitrile derivative was dissolved in 4 N HCl in ethyl acetate solution (20 mL), and diethyldithiophosphate (3.17 mL, 18.9 mmol) was added to this solution. The reaction mixture was stirred for 6 h at room temperature. The

obtained precipitate was collected and washed with ethyl acetate and ethyl ether. Phthalimido-thioacetamide **5b** was obtained (2.29 g, 54%): ^{1}H NMR (DMSO-d_6) δ 4.46 (2H, s), 7.85–7.98 (4H, m), 9.44 (1H, brs), 9.76 (1H, brs); EI-MS *m*/*e* (M^+) 220.

2-Phthalimidopropanethioamide (5c). Compound **5c** was synthesized from 4-phthalimidopropionitrile according to the same procedure as that for **5b** (yield quant): ^{1}H NMR (DMSO-d_6) δ 2.82 (2H, t, J = 7.6 Hz), 3.92 (2H, t, J = 6.8 Hz), 7.85 (4H, s), 9.37 (2H, brs); EI-MS *m*/*e* (M^+) 234.

3-Phthalimidobutylthioamide (5d). Compound **5d** was synthesized from 3-phthalimidobutyronitrile according to the same procedure as that for **5b** (yield quantitative): ^{1}H NMR (DMSO-d_6) δ 1.97–2.06 (2H, m), 2.42–2.55 (2H, m), 3.60 (2H, t, J = 7.1 Hz), 7.86 (4H, s), 9.20–9.40 (2H, br); EI-MS *m*/*e* (M^+) 248.

4-Phthalimidovarerothioamide (5e). Compound **5e** was synthesized from 4-phthalimidovareronitrile according to the same procedure as that for **5b** (yield quantitative): ^{1}H NMR (DMSO-d_6) δ 1.62 (4H, m), 2.50 (2H, t, J = 6.5 Hz), 3.58 (2H, t, J = 6.5 Hz), 7.85 (4H, m), 9.30 (2H, br); EI-MS *m*/*e* (M^+) 262.

2-Phthalimidomethyl-8H-indeno[1,2-*d*]thiazole (6b). A solution of **5b** (2.00 g, 7.80 mmol) and 2-bromo-1-indanone (1.64 g, 7.80 mmol) in 2-propanol (100 mL) was stirred under reflux for 6 h. The precipitate was collected and dissolved in $CHCl_3$, which was washed with saturated aqueous $NaHCO_3$, H_2O, brine and dried over $MgSO_4$, then concentrated in vacuo to afford **6b** (1.30 g, 61%): ^{1}H NMR (DMSO-d_6) δ 3.79 (2H, s), 5.29 (2H, s), 7.20–7.51 (4H, m), 7.70–7.98 (4H, m); FAB-MS *m*/*e* ($M^+ + 1$) 333.

2-(2-Phthalimidoethyl)-8H-indeno[1,2-*d*]thiazole (6c). Compound **6c** was synthesized from **5c** according to the same procedure as that for **6b** (yield 70%): ^{1}H NMR ($CDCl_3$) δ 3.49(2H, t, J = 7.4 Hz), 3.78 (2H, s), 4.18 (2H, t, J = 6.6 Hz), 7.19–7.90 (8H, m); EI-MS *m*/*e* (M^+) 346.

2-(3-Phthalimidopropyl)-8H-indeno[1,2-*d*]thiazole (6d). Compound **6d** was synthesized from **5d** according to the same procedure as that for **6b** (yield 49%): ^{1}H NMR ($CDCl_3$) δ 2.21–2.45 (2H, m), 3.19 (2H, t, J = 7.4 Hz), 3.78 (2H, s), 3.87 (2H, t, J = 7.1 Hz), 7.19–7.51 (4H, m), 7.60–7.87 (4H, m); EI-MS *m*/*e* (M^+) 360.

2-(3-Phthalimidobutyl)-8H-indeno[1,2-*d*]thiazole (6e). Compound **6e** was synthesized from **5e** according to the same procedure as that for **6b** (yield 28%): ^{1}H NMR ($CDCl_3$) δ 1.74–1.83 (4H, m), 3.05–3.28 (2H, m), 3.70–3.77 (2H, m), 3.78 (2H, s), 7.69–7.80 (8H, m); EI-MS *m*/*e* (M^+) 374.

2-Amino-8H-indeno[1,2-*d*]thiazole (7a). A solution of 2-bromo-1-indenone (1.93 g, 9.14 mmol) and thiourea (696 mg, 9.14 mmol) in ethanol (20 mL) was stirred under reflux for 4 h. The precipitate was collected and dissolved in $CHCl_3$, which was washed with saturated aqueous $NaHCO_3$, H_2O, brine and dried over $MgSO_4$, and then concentrated in vacuo to afford the crude aminoindenothiazole. This oil was dissolved in methanol and treated with fumaric acid; **6a** was obtained as a hemifumarate (0.10 g, 4.4% yield): ^{1}H NMR (DMSO-d_6) δ 3.68 (2H, s), 6.64 (1H, s), 7.14 (1H, t, J = 7.8 Hz), 7.28 (1H, t, J = 7.3 Hz), 7.46 (1H, d, J = 7.3 Hz); EI-MS *m*/*e* (M^+) 188; Anal. calcd for $C_{10}H_8N_2S{\cdot}0.5C_4H_4O_4$: C,58.52; H, 4.09; N, 11.37; S, 13.02. Found: C, 58.38; H, 4.06; N, 11.28; S, 13.10; mp 220–223 °C.

2-Aminomethyl-8H-indeno[1,2-d]thiazole (7b). To a solution of **6b** (1.30 g, 3.90 mmol) in methanol (10 mL) was added 40% methylamine in methanol (20 mL). The reaction mixture was stirred for 16 h at room temperature. The reaction mixture was concentrated in vacuo; then the residue was dissolved in $CHCl_3$ and the organic layer was extracted with aqueous 1 N HCl. The organic layer was separated and discarded. The aqueous layer was basified with aqueous 1 N NaOH and extracted with $CHCl_3$. The organic layer was washed with H_2O, brine and dried over Na_2SO_4, and then concentrated in vacuo to afford the crude 2-aminomethyl-8H-indeno[1,2-*d*]thiazole. This oil was dissolved in methanol and treated with fumaric acid. **7b** was obtained as a fumarate (730 mg, yield 59%): ^{1}H NMR (DMSO-d_6) δ 3.93 (2H, s), 4.28 (2H, s), 6.55 (2H, s), 7.26 (1H, t, J = 6.7 Hz), 7.37 (1H, t, J = 7.3 Hz), 7.57 (1H, d, J = 7.3 Hz), 7.63 (1H, d, J = 7.3 Hz); EI-MS *m*/*e* (M+) 202; Anal. calcd for $C_{11}H_{10}N_2S{\cdot}C_4H_4O_4{\cdot}0.1H_2O$: C,56.27; H, 4.47; N, 8.75; S, 10.02. Found: C, 56.12; H, 4.45; N, 8.62; S, 10.12; mp 184–186 °C.

2-(2-Aminoethyl)-8H-indeno[1,2-*d*]thiazole (7c). Compound **7c** was synthesized from **6c** according to the same procedure as that for **7b**. Compound **7c** was obtained as a fumarate (yield 64%): ^{1}H NMR (DMSO-d_6) δ 3.23 (2H, t, J = 7.3 Hz), 3.36 (2H, t, J = 7.3 Hz), 3.92 (2H, s), 6.45 (2H, s), 7.27 (1H, t, J = 7.3 Hz), 7.37 (1H, t, J = 7.3 Hz), 7.57 (1H, d, J = 7.3 Hz), 7.66 (1H, d, J = 7.3 Hz); EI-MS *m*/*e* (M^+) 216; Anal. calcd for $C_{12}H_{12}N_2S{\cdot}C_4H_4O_4$: C,57.82; H, 4.85; N, 8.43; S, 9.65. Found: C, 57.48; H, 4.91; N, 8.38; S, 9.62; mp 208–210 °C.

2-(3-Aminopropyl)-8H-indeno[1,2-*d*]thiazole (7d). Compound **7d** was synthesized from **6d** according to the same procedure as that for **7b**. Compound **7d** was obtained as a fumarate (yield quantitative): ^{1}H NMR (DMSO-d_6) δ 2.03–2.11 (2H, m), 2.90 (2H, t, J = 6.8 Hz), 3.17 (2H, t, J = 7.3 Hz), 3.91 (2H, s), 6.42 (2H, s), 7.26 (1H, t, J = 7.3 Hz), 7.37 (1H, t, J = 7.3 Hz), 7.56 (1H, d, J = 7.3 Hz), 7.63 (1H, d, J = 7.3 Hz); EI-MS *m*/*e* (M^+) 230; Anal. calcd for $C_{13}H_{14}N_2S{\cdot}C_4H_4O_4$: C,58.94; H, 5.24; N, 8.09; S, 9.26. Found: C, 58.73; H, 5.17; N, 8.05; S, 9.20; mp 178–180 °C.

2-(4-Aminobutyl)-8H-indeno[1,2-*d*]thiazole (7e). Compound **7e** was synthesized from **6e** according to the same procedure as that for **7b**. Compound **7e** was obtained as a fumarate (yield 32%): ^{1}H NMR (DMSO-d_6) δ 1.62–1.70 (2H, m), 1.80–1.87 (2H, m), 2.82 (2H, t,

$J=7.3$ Hz), 3.10 (2H, t, $J=7.3$ Hz), 3.90 (2H, s), 6.41 (2H, s), 7.25 (1H, t, $J=7.3$ Hz), 7.36 (1H, t, $J=7.3$ Hz), 7.56 (1H, d, $J=7.3$ Hz), 7.62 (1H, d, $J=7.3$ Hz); EI-MS m/e (M^+) 244; Anal. calcd for $C_{14}H_{16}N_2S{\cdot}C_4H_4O_4$: C,59.68; H, 5.62; N, 7.73; S, 8.85. Found: C, 59.50; H, 5.73; N, 7.62; S, 8.87; mp 183–186 °C.

General procedure for synthesis of the 2-substituted-indeno[1,2-*d*]thiazoles

2-(2-Dimethylaminoethyl)-8H-indeno[1,2-*d*]thiazole (7f). A solution of 2-bromo-1-indenone (630 mg, 2.98 mmol), 3-dimethylaminopropanethioamide hydrochloride (500 mg, 2.98 mmol) in 2-propanol (10 mL) was stirred under reflux for 1.5 h. The precipitate was collected and dissolved in $CHCl_3$, which was washed with saturated aqueous $NaHCO_3$, H_2O, brine and dried over $MgSO_4$, and then concentrated in vacuo to afford the crude aminoindenothiazole. This oil was dissolved in methanol and treated with fumaric acid. **7f** was obtained as a fumarate (226 mg, 21% yield): ^{1}H NMR (DMSO-d_6) δ 2.35 (6H, s), 2.86 (2H, t), 3.23 (2H, t), 3.90 (2H, s), 6.59 (2H, s), 7.25 (1H, t, $J=7.3$ Hz), 7.36 (1H, t, $J=7.8$ Hz), 7.55 (1H, d, $J=7.3$ Hz), 7.62 (1H, d, $J=7.3$ Hz); FAB-MS m/e ($M^+ + 1$) 245; mp 142–144 °C.

The following compounds were synthesized using the above general procedure.

2-(3-Dimethylaminopropyl)-8H-indeno[1,2-*d*]thiazole (7g). Compound **7g** was obtained as a sesqui fumarate (yield 47%): ^{1}H NMR (DMSO-d_6) δ 2.10 (2H, m), 2.59 (6H, s), 2.91 (2H, t, $J=7.8$ Hz), 3.13 (2H, t, $J=7.8$ Hz), 3.91 (2H, s), 6.56 (3H, s), 7.14 (1H, t, $J=7.8$ Hz), 7.28 (1H, t, $J=7.3$ Hz), 7.34 (1H, d, $J=7.3$ Hz), 7.46 (1H, d, $J=7.3$ Hz); EI-MS m/e (M^+) 258; Anal. calcd for $C_{15}H_{18}N_2S{\cdot}1.5C_4H_4O_4$: C,57.84; H, 5.64; N, 6.42; S, 7.35. Found: C, 57.71; H, 5.55; N, 6.41; S, 7.59; mp 123–125 °C.

2-[(1-Methyl-3-pyrrolidinyl)methyl]-8H-indeno[1,2-*d*]thiazole (8b). Compound **8b** was obtained as a fumarate (yield 59%): ^{1}H NMR (DMSO-d_6) δ 1.65–1.74 (1H, m), 2.06–2.14 (1H, m), 2.56 (3H, s), 2.74–2.82 (2H, m), 2.91–3.03 (2H, m), 3.09–3.15 (1H, m), 3.19–3.23 (2H, m), 3.91 (2H, s), 6.52 (2H, s), 7.24 (1H, t, $J=7.3$ Hz), 7.37 (1H, t, $J=7.3$ Hz), 7.56 (1H, d, $J=7.3$ Hz), 7.64 (1H, d, $J=7.3$ Hz); FAB-MS m/e ($M^+ + 1$) 271; Anal. calcd for $C_{16}H_{18}N_2S{\cdot}C_4H_4O_4$: C, 62.16; H, 5.74; N, 7.25; S, 8.30. Found: C, 62.11; H, 5.69; N, 7.22; S, 8.31; mp 142–143 °C.

2-[(1-Methyl-2-pyrrolidinyl)methyl]-8H-indeno[1,2-*d*]thiazole (8c). Compound **8c** was obtained as a fumarate (yield 72%): ^{1}H NMR (DMSO-d_6) δ 1.59–1.77 (3H, m), 1.92–1.99 (1H, m), 2.44–2.54 (4H, m), 2.97–3.05 (1H, m), 3.15–3.24 (2H, m), 3.48–3.53 (1H, m), 3.91 (2H, s), 6.58 (2H, s), 7.23–7.27 (1H, m), 7.34–7.38 (1H, m), 7.56 (1H, d, $J=7.3$ Hz), 7.64 (1H, d, $J=7.3$ Hz); FAB-MS m/e ($M^+ + 1$) 271; Anal. calcd for $C_{16}H_{18}N_2S{\cdot}C_4H_4O_4{\cdot}0.2H_2O$: C,61.58; H, 5.79; N, 7.18; S, 8.22. Found: C, 61.59; H, 5.69; N, 7.25; S, 8.17; mp. 172 °C(dec).

2-[2-(1-Methyl-2-pyrrolidinyl)ethyl]-8H-indeno[1,2-*d*]thiazole (8d). Compound **8d** was obtained as a fumarate (yield 30%): ^{1}H NMR (DMSO-d_6) δ 1.62–1.71 (1H, m), 1.81–1.89 (2H, m), 1.91–1.98 (2H, m), 2.11–2.19 (1H, m), 2.30–2.36 (1H, m), 2.53 (3H, s), 2.65–2.69 (1H, m), 2.87 (1H, br), 3.09–3.24 (2H, m), 3.34–3.37 (1H, m), 3.93 (2H, s), 6.57 (2H, s), 7.28 (1H, t, $J=7.3$ Hz), 7.39 (1H, t, $J=7.3$ Hz), 7.58 (1H, d, $J=7.8$ Hz), 7.66 (1H, d, $J=7.3$ Hz); EI-MS m/e (M+) 284; Anal. calcd for $C_{17}H_{20}N_2S{\cdot}C_4H_4O_4{\cdot}0.4H_2O$: C, 61.87; H, 6.13; N, 6.87; S, 7.87. Found: C, 61.84; H, 6.08; N, 6.82; S, 7.84; mp 138–139 °C.

2-(3-Quinuclidinyl)-8H-indeno[1,2-*d*]thiazole (8e). Compound **8e** was obtained as a fumarate (yield 26%): ^{1}H NMR (DMSO-d_6) δ 1.62–1.99 (4H, m), 2.29–2.30 (1H, m), 3.02–3.17 (4H, m), 3.53–3.75 (3H, m), 3.94 (2H, s), 6.52 (2H, s), 7.27 (1H, t, $J=7.3$ Hz), 7.35 (1H, t, $J=7.3$ Hz), 7.51 (1H, d, $J=7.8$ Hz), 7.69 (1H, d, $J=7.3$ Hz); FAB-MS m/e ($M^+ + 1$) 283; Anal. calcd for $C_{17}H_{18}N_2S{\cdot}C_4H_4O_4$: C,63.30; H, 5.56; N, 7.03; S, 8.05. Found: C, 63.50; H, 5.67; N, 6.95; S, 7.95; mp 199–202 °C.

5-[(8H-Indeno[1,2-*d*]thiazol-2-yl)methyl]-1-azabicyclo[3,3,0]octane (8f). Compound **8f** was obtained as a fumarate (yield 22%): ^{1}H NMR (DMSO-d_6) δ 1.79–1.85 (2H, m), 2.00–2.03 (4H, m), 2.31–2.36 (2H, m), 2.87–2.92 (2H, m), 3.52 (2H, s), 3.57–3.62 (2H, m), 3.84 (2H, s), 6.69 (2H, s), 7.24 (1H, t, $J=7.3$ Hz), 7.35 (1H, t, $J=7.3$ Hz), 7.51 (1H, d, $J=7.8$ Hz), 7.69 (1H, d, $J=7.3$ Hz); FAB-MS m/e ($M^+ + 1$) 297; Anal. calcd for $C_{18}H_{20}N_2S{\cdot}C_4H_4O_4$: C,64.06; H, 5.86; N, 6.79; S, 7.77. Found: C, 63.79; H, 5.89; N, 6.74; S, 7.79; mp 201–202 °C.

2-(1-Methyl-2-oxo-4-pyrrolidinyl)-8H-indeno[1,2-*d*]thiazole (9). Compound **9** was obtained as a white powder (yield 36%): ^{1}H NMR (DMSO-d_6) δ 2.64 (1H, dd, $J=6.7$ Hz, 16.5 Hz), 2.79 (3H, s), 2.84 (1H, dd, $J=6.7$ Hz, 16.5 Hz), 3.64 (1H, dd, $J=6.1$ Hz, 9.7 Hz), 3.87 (1H, dd, $J=8.5$ Hz, 9.7 Hz), 3.94 (2H, s), 4.09–4.15 (1H, m), 7.26 (1H, t, $J=7.3$ Hz), 7.38 (1H, t, $J=7.3$ Hz), 7.59 (1H, d, $J=7.8$ Hz), 7.66 (1H, d, $J=7.3$ Hz); EI-MS m/e (M^+) 270; mp 114–116 °C.

2-(2-Pyridylmethyl)-8H-indeno[1,2-*d*]thiazole (10a). Compound **10a** was obtained as a fumarate (yield 6.5%): ^{1}H NMR (DMSO-d_6) δ 3.89 (2H, s), 4.59 (2H, s), 6.64 (2H, s), 7.24 (1H, dd, $J=6.3$ Hz, 7.2 Hz), 7.26–7.38 (2H, m), 7.46 (1H, d, $J=7.8$ Hz), 7.55 (1H, d, $J=7.8$ Hz), 7.63 (1H, d, $J=7.8$ Hz), 7.79 (1H, dt, $J=2.0$ Hz,7.8 Hz), 8.54 (1H, d, $J=4.8$ Hz), 13.15 (2H, brs); EI-MS m/e (M+) 264; Anal. calcd for $C_{16}H_{12}N_2S{\cdot}C_4H_4O_4$: C,63.15; H, 4.24; N, 7.36; S, 8.43. Found: C, 63.09; H, 4.18; N, 7.33; S, 8.50; mp 158–160 °C.

2-(3-Pyridylmethyl)-8H-indeno[1,2-*d*]thiazole (10b). Compound **10b** was obtained as a pale yellow crystal (yield 25%): ^{1}H NMR ($CDCl_3$) δ 3.79 (2H, s), 4.44 (2H, s), 7.25–7.28 (2H, m), 7.39 (1H, d, $J=7.8$ Hz), 7.48 (1H, d, $J=7.8$ Hz), 7.69 (1H, d, $J=7.8$ Hz), 7.74 (1H, d, $J=7.8$ Hz), 8.54 (1H, dd, $J=1.5$ Hz, 4.9 Hz), 8.65 (1H, $J=2.0$ Hz); EI-MS m/e (M+) 264; Anal. calcd for

$C_{16}H_{12}N_2S$: C,72.70; H, 4.58; N, 10.60; S, 12.13. Found: C, 72.71; H, 4.59; N, 10.61; S, 12.31; mp 165–167 °C.

2-(4-Pyridylmethyl)-8H-indeno[1,2-*d*]thiazole (10c). Compound **10c** was obtained as a pale yellow crystal (yield 20%): ^{1}H NMR ($CDCl_3$) δ 3.81 (2H, s), 4.43 (2H, s), 7.23–7.29 (3H, m), 7.38 (1H, t, J = 7.3 Hz), 7.49 (1H, d, J = 7.8 Hz), 7.78 (1H, d, J = 7.3 Hz), 8.57 (2H, d, J = 5.5 Hz); EI-MS m/e (M+) 264; Anal. calcd for $C_{16}H_{12}N_2S$: C,72.70; H, 4.58; N, 10.60; S, 12.13. Found: C, 72.63; H, 4.59; N, 10.43; S, 11.96; mp 122–123 °C.

2-(1H-Imidazol-2-ylmethyl)-8H-indeno[1,2-*d*]thiazole (10d). Compound **10d** was obtained as a fumarate (yield 4.1%): ^{1}H NMR (DMSO-d_6) δ 3.89 (2H, s), 4.47 (2H, s), 6.63 (2H, s), 7.25 (1H, t, J = 7.3 Hz), 7.36 (1H, t, J = 7.3 Hz), 7.56 (1H, d, J = 7.8 Hz), 7.63 (1H, d, J = 7.3 Hz); FAB-MS m/e (M^+ + 1) 254; Anal. calcd for $C_{17}H_{20}N_2S{\cdot}C_4H_4O_4$: C,62.98; H, 6.04; N, 6.99; S, 8.01. Found: C, 62.88; H, 6.08; N, 7.04; S, 8.16; mp 201–204 °C.

2-(1-Imidazolylmethyl)-8H-indeno[1,2-*d*]thiazole (10e). Compound **10e** was obtained as a fumarate (yield 20%): ^{1}H NMR (DMSO-d_6) δ 3.93 (2H, s), 5.69 (2H, s), 6.63 (2H, s), 6.98 (1H, s), 7.28 (1H, t, J = 7.8 Hz), 7.33 (1H, s), 7.38 (1H, t, J = 7.3 Hz), 7.57 (1H, d, J = 7.3 Hz), 7.66 (1H, d, J = 7.3 Hz), 7.89 (1H, s); EI-MS m/e (M^+) 253; Anal. calcd for $C_{14}H_{11}N_3S{\cdot}C_4H_4O_4{\cdot}0.1H_2O$: C,58.24; H, 4.13; N, 11.32; S, 8.64. Found: C, 58.21; H, 4.09; N, 11.05; S, 8.74; mp 155–159 °C.

2-(1H-imidazol-4-ylmethyl)-8H-indeno[1,2-*d*]thiazole (10f). Compound **10f** was obtained as a fumarate (yield 32%): ^{1}H NMR (DMSO-d_6) δ 3.87 (2H, s), 4.34 (2H, s), 6.63 (2H, s), 7.07 (1H, s), 7.24 (1H, t, J = 7.8 Hz), 7.36 (1H, t, J = 7.3 Hz), 7.54 (1H, d, J = 7.8 Hz), 7.63 (1H, d, J = 7.3 Hz), 7.69 (1H, s); EI-MS m/e (M+) 253; Anal. calcd for $C_{14}H_{11}N_3S{\cdot}C_4H_4O_4$: C, 58.53; H, 4.09; N, 11.38; S, 8.68. Found: C, 58.37; H, 4.21; N, 11.25; S, 8.69; mp 202–203 °C.

2-[2-(1H-imidazol-2-yl)ethyl]-8H-indeno[1,2-*d*]thiazole (10g). Compound **10g** was obtained as a fumarate (yield 5.9%): ^{1}H NMR (DMSO-d_6) δ 3.15 (2H, t, J = 7.8 Hz), 3.48 (2H, t, J = 7.8 Hz), 3.89 (2H, s), 6.62 (2H, s), 6.94 (2H, s), 7.25 (1H, t, J = 7.8 Hz), 7.36 (1H, t, J = 7.3 Hz), 7.54(1H, d, J = 7.8 Hz), 7.63 (1H, d, J = 7.3 Hz); EI-MS m/e (M+) 267; Anal. calcd for $C_{15}H_{13}N_3S{\cdot}C_4H_4O_4{\cdot}0.1H_2O$: C,59.24; H, 4.50; N, 10.91; S, 8.31. Found: C, 58.00; H, 4.21; N, 10.31; S, 7.80; mp 177–180 °C.

2-[2-(1H-Imidazol-1-yl)ethyl]-8H-indeno[1,2-*d*]thiazole (10h). Compound **10h** was obtained as a white powder (yield 24%): ^{1}H NMR (DMSO-d_6) δ 3.57 (2H, t, J = 7.3 Hz), 3.89 (2H, s), 4.49 (2H, t, J = 7.3 Hz), 7.26 (1H, t, J = 3.9 Hz), 7.27 (1H, t, J = 7.8 Hz), 7.37 (1H, t, J = 7.3 Hz), 7.56 (1H, d, J = 7.3 Hz), 7.64 (1H, d, J = 7.3 Hz), 7.71 (s, 1H); EI-MS m/e (M+) 267; Anal. calcd for $C_{15}H_{13}N_3S{\cdot}0.4H_2O$: C,65.62; H, 5.07; N, 15.30; S, 11.68. Found: C, 65.74; H, 4.90; N, 15.13; S, 11.60; mp 118–121 °C.

2-[2-(1H-Imidazol-4-yl)ethyl]-8H-indeno[1,2-*d*]thiazole (10i). Compound **10i** was obtained as a fumarate (yield 23%): ^{1}H NMR (DMSO-d_6) δ 3.03 (2H, t, J = 7.3 Hz), 3.39 (2H, t, J = 7.4 Hz), 3.89 (2H, s), 6.62 (3H, s), 6.88 (1H, s), 7.25 (1H, t, J = 6.4 Hz), 7.36 (1H, t, J = 6.8 Hz), 7.56 (1H, d, J = 7.3 Hz), 7.62 (1H, d, J = 7.8 Hz), 7.70 (1H, s); EI-MS m/e (M+) 267; mp 143–147 °C.

2-(1-Methyl-3-pyrrolidinyl)-8H-indeno[1,2-*d*]thiazole (8a). A solution of compound **9** (1.54 g, 5.70 mmol) in toluene (70 mL) was added a Red-Al® (4.1 mL) was added with dropwise at 0 °C. This solution was warmed up to room temperature in 30 min, then stirred under reflux for 2 h. The reaction mixture was cooled to 0 °C, and added aqueous 1 N NaOH solution (30 mL) then stirred for 30 min at room temperature. Organic layer was separated, then washed with brine, dried over $MgSO_4$ then, concentrated in vacuo. The residue was purified by column chromatography on silica gel eluting with 50:1:0.1 $CHCl_3$/methanol/29% aqueous ammonia. Obtained free amine was dissolved in methanol and treated with fumaric acid. **8a** was obtained as a fumarate (226 mg, 21% yield): ^{1}H NMR (DMSO-d_6) δ 2.09–2.13 (1H, m), 2.38–2.47 (4H, m), 2.74 (1H, m), 3.10 (1H, m), 3.87–3.93 (3H, m), 6.59 (2H, s), 7.25 (1H, t, J = 6.4 Hz), 7.36 (1H, t, J = 6.8 Hz), 7.56 (1H, d, J = 7.3 Hz), 7.63 (1H, d, J = 7.8 Hz); EI-MS m/e (M^+) 256; Anal. calcd for $C_{15}H_{16}N_2S{\cdot}C_4H_4O_4{\cdot}H_2O$: C,60.98; H, 5.44; N, 7.49; S, 8.57. Found: C, 60.80; H, 5.35; N, 7.48; S, 8.30; mp 129–131 °C.

Biology

Contractile effects in the isolated guinea pig colon.[19] The distal portion of the colon was removed from a Hartley guinea pig (300–500 g). The colon was cleaned in fresh Krebs-bicarbonate buffer at room temperature and cut into approximately 20-mm segments. The segments were suspended longitudinally in an organ bath containing Krebs-bicarbonate solution warmed to 37 °C and equilibrated with 95% O_2/5% CO_2. Isometric contraction under a loading tension of 1 g was recorded. The agonists were applied cumulatively to the bath. For antagonist studies, antagonists were added to the bath 15 min before the application of the agonists. For the desensitization of 5-HT_3 receptors, 2-methyl-5-HT (30 μM) was added to the bath 30 min before the application of the agonists. Activity of each compound is expressed by a relative value in comparison with the activity of 5-HT in each specimen. The intrinsic activity (relative efficacy compared to 5-HT) is indicated as percentage of the maximum response by each compound when the maximum contraction by 5-HT is defined as 100%. The relative potency compared to 5-HT is determined according to the following equation; relative potency = EC_{50} value for the 5-HT/EC_{50} value for a compound. The contractile effect of each compound was antagonized by 0.3 μM ramosetron which is a selective 5-HT_3 receptor antagonist.

Radioligand 5-HT_3 receptor binding studies.[21] The cloned rat 5-HT_3 receptor was expressed on COS-1 cells by transfection of a plasmid. The transfected cells were

homogenized in HEPES buffer and centrifuged at 48,000 *g* for 10 min. Membranes were incubated with [^{3}H]ramosetron and inhibitor for 30 min at 25 °C. The incubation was terminated by filtration and washing with HEPES buffer through GF/B filters. Radioactivity of the filter was counted with Top Count. IC_{50} values were calculated by logit-log analysis.

Defecation in normal ferrets.[23] Male ferrets weighing 0.8–1.5 kg ($n = 60$) were used. The animals were fed an ordinary laboratory chow and allowed free access to water under a constant 12 h light-dark cycle. The food was given once daily in the middle of the light cycle (1:00 pm). The animals were used repeatedly with 1 week washout periods between the different tests. All experiments were performed in compliance with the regulations of the Animal Ethical Committee of Yamanouchi Pharmaceutical. **10f** was administered orally at 10:00 am and defecation, emetic episodes including vomiting and retching, and some other behavioral changes were observed. In the preliminary observations on the duration of the effects of these compounds on defecation, the effect of **10f** finished within 2 h after administration. We observed, therefore, the effects of **10f** for 2 h, respectively. No food was supplied during the observation. In the antagonist study, ramosetron 0.1 μg/kg was administered subcutaneously 0.5 h before the administration of **10f**. The stools were collected and dried (110 °C, 24 h). The water content was estimated by measuring both wet and dry weights of stools. In the case of repeated dosing, **10f** was orally administered once daily at 10:00 am for 5 days, and the frequency of defecation for 1 h after administration was observed each day.

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References and Notes

1. Koch, T. R. In *Bockus Gastroenterology*. 5th ed.; Haubrich W. S., Shaffner F., Eds; Saunders: Philadelphia, 1995; Vol. I, p 102.
2. (a) Erspamer V. In *Handbuch der experimentellen Pharmacologie*; Eichler O., Farah A., Eds; Springer: Berlin, 1966; Vol. 19, p 132. (b) Wood J.D. In *Physiology of the Gastrointestinal Tract*. *3rd ed*.; Johnson L. R., Ed.; Raven: New York, 1994; Vol. I, p 423.
3. (a) Mawe, G. M.; Branchek, T. A.; Gershon, M. D. *Proc. Natl. Acad. Sci. U.S.A*. **1986**, *83*, 9799. (b) Ito, H.; Tamura, K. *Eur. J. Pharmacol*. **1995**, *283*, 107.
4. Miyata, K.; Kamato, T.; Nishida, A.; Ito, H.; Yuki, H.; Yamano, M.; Tsutsumi, R.; Katsuyama, Y.; Honda, K. *J. Pharmacol. Exp. Ther*. **1992**, *261*, 297.
5. (a) Kamato, T.; Ito, H.; Nagakura, Y.; Yamano, M.; Miyata, K. *Jpn. J. Pharmacol*. **1992**, *58*, 128. (b) Nagakura, Y.; Kamato, T.; Nishida, A.; Ito, H.; Yamano, M.; Miyata, K. *Naunyn- Scmiedeberg's Arch. Pharmacol*. **1996**, *353*, 489.
6. Tally, N. J.; Phillips, S. F.; Haddad, A.; Miller, L. J.; Twomey, C.; Zinsmeister, A. R.; MacCarty, R. L.; Ciociola, A. *Dig. Dis. Sci*. **1990**, *35*, 477 i.
7. Camilleri, M.; Mayer, E. A.; Drossman, D. A.; Heath, A.; Duke, G. E.; McSorley, D.; Kongs, S.; Mangel, A. W.; Northcutt, A. R. *Aliment. Pharmacol. Ther*. **1999**, *13*, 1149.
8. Richardson, B. P.; Engrl, G.; Donatsch, P.; Stadler, P. A. *Nature* **1985**, *316*, 126.
9. Kilpatrick, G. H.; Butler, A.; Burridge, J.; Oxford, A. W. *Eur. J. Pharmacol*. **1990**, *182*, 193.
10. Nagel, A. A.; Rosen, T.; Rizzi, J.; Daffeh, J.; Guarino, K.; Nowakowski, J.; Vincent, L. A.; Heym, J.; McLean, S.; Seeger, T.; Connolly, M.; Schmid, A. W.; Siok, C. *J. Med. Chem*. **1990**, *33*, 13.
11. Bradley, G.; Ward, T. J.; White, J. C.; Coleman, J.; Taylor, A.; Rhodes, K. F. *J. Med. Chem*. **1992**, *35*, 1515.
12. Bermudez, J.; Boyland, P.; King, F. D.; Summersell, R. J. *Bioorg. Med. Chem. Lett*. **1993**, *3*, 205.
13. Bachy, A.; Héaulme, M.; Giudice, A.; Michaud, J-C.; Lefevre, I. A.; Souilhac, J.; Manara, L.; Emerit, M. B.; Gozlan, H.; Hamon, M.; Keane, P. E.; Soubrié, P.; Fur, G. L. *Eur. J. Pharmacol*. **1993**, *237*, 299.
14. Perrone, R.; Berardi, F.; Colabufo, N. A.; Tortorella, V.; Lograno, M. D.; Daniele, E.; Govoni, S. *Il Farmaco* **1995**, *50*, 77.
15. Clark, R. D.; Muchowski, J. M.; Weinhardt, K. K.; Dillon, M. P.; Lee, C.-H.; Bley, K. R.; Bonhaus, D. W.; Wong, E. H. F.; Eglen, R. M. *Bioorg. Med. Chem. Lett*. **1995**, *5*, 1853.
16. Sato, Y.; Yamada, M.; Yoshida, S.; Soneda, T.; Ishikawa, M.; Nizato, T.; Suzuki, K.; Konno, F. *J. Med. Chem*. **1998**, *41*, 3015.
17. Fozord, J. R.; Host, M. *Br. J. Pharmacol*. **1982**, *77*, 520.
18. Ohta M., Yanagisawa I., Miyata K., *International Patent Application* WO92/07849, May 14, 1992.
19. Ito, H.; Kiso, T.; Miyata, K.; Kamato, T.; Yuki, H.; Akuzawa, S.; Nagakura, Y.; Yamano, M.; Suzuki, M.; Naitoh, Y.; Sakai, H.; Iwaoka, K.; Yamaguchi, T. *Eur. J. Pharmcol*. **2000**, *409*, 195.
20. Miyata, K.; Kamato, T.; Nishida, A.; Ito, H.; Katsuyama, Y.; Iwai, A.; Yuki, H.; Yamano, M.; Tsutsumi, R.; Ohta, M.; Takeda, M.; Honda, K. *J. Pharmacol. Exp. Ther*. **1991**, *259*, 15.
21. Miyake, A.; Mochizuki, S.; Takemoto, Y.; Akuzawa, S. *Mol. Pharmacol*. **1995**, *48*, 407.
22. Davies, P. A.; Pistis, M.; Hanna, M. C.; Peters, J. A.; Lambert, J. J.; Hales, T. G.; Kirkness, E. F. *Nature* **1999**, *397*, 359.
23. Ito, H.; Kiso, T.; Yuki, H.; Naitoh, Y.; Miyata, K.; Iwaoka, K.; Yamaguchi, T. *Eur. J. Pharmcol*. **2001**, *424*, 151.