Novel 5-Hydroxytryptamine (5-HT$_3$) Receptor Antagonists. Synthesis and Structure–Activity Relationships of 9-Methyl-2,3,4,9-tetrahydrothiopyrano[2,3-*b*]indol-4-one Derivatives

Takeshi SUZUKI, Akira MATSUHISA, Keiji MIYATA, Isao YANAGISAWA, and Mitsuaki OHTA*

Neuroscience/Gastrointestinal Research Laboratories, Institute for Drug Discovery Research, Yamanouchi Pharmaceutical Co., Ltd., 21 Miyukigaoka, Tsukuba, Ibaraki 305, Japan.
Received May 15, 1996; accepted September 20, 1996

Novel 9-methyl-4,9-dihydrothiopyrano[2,3-*b*]indol-4-one derivatives 2b—e, 3-methylene-9-methyl-2,3,4,9-tetrahydrothiopyrano[2,3-*b*]indol-4-one derivatives 3b—e and 9-methyl-2,3,4,9-tetrahydrothiopyrano[2,3-*b*]indol-4-one derivatives 4a—e were prepared. The 5-hydroxytryptamine (5-HT$_3$) receptor-antagonistic activities of these compounds were evaluated by using the von Bezold–Jarisch reflex test (B. J. reflex, rats) and the contractile response to 5-HT in the isolated distal colon (guinea pig). The 5-ethyl-4-imidazolyl derivative 4d was found to be 79 times more potent than ondansetron 1 in the B. J. reflex test (ID_{50} = 0.048 μg/kg, i.v.), and the 5-methyl-4-imidazolyl derivative 4c was found to be 126 times more potent than 1 in the colonic contraction (IC_{50} = 0.0062 μM) assay.

Key words thiopyrano[2,3-*b*]indole; 5-HT$_3$ receptor antagonist; irritable bowel syndrome; vomiting; structure–activity relationship

5-Hydroxytryptamine (5-HT) is a biogenic amine that mediates a variety of physiological actions. 5-HT receptors have been pharmacologically classified into at least four subtypes, 5-HT$_1$, 5-HT$_2$, 5-HT$_3$ and 5-HT$_4$,[1] among which the 5-HT$_3$ receptor has been shown to be a ligand-gated ion channel which causes fast and depolarizing responses in neuronal cells[2] and to be located in both the central and peripheral nervous systems.[3] In the last decade, research on antagonists for the 5-HT$_3$ receptor has accelerated, in view of the possible roles of such antagonists as therapeutic agents for the treatment of nausea and vomiting associated with cancer chemotherapy, pain of vascular origin, disorders of the central nervous system, and gastrointestinal disorders.[4] Some 5-HT$_3$ receptor antagonists, *e.g.*, ondansetron **1**,[5] are already in clinical use as effective agents in suppressing the vomiting often associated with cancer chemotherapy.

Endogenous 5-HT is located in the blood, the nervous system and the gut, and approximately 90% of it is estimated to be within the gastrointestinal tract. The role of 5-HT$_3$ receptors in controlling gastrointestinal contractility *in vitro* (isolated guinea pig ileum[5,6] or colon[7]) is well established. Furthermore, our group has demonstrated that 5-HT may mediate bowel dysfunction related to stress *via* 5-HT$_3$ receptors, based on the results of *in vivo* tests in rodents.[8] In humans, stress commonly results in gastrointestinal disorders such as irritable bowel syndrome (IBS).[9,10] The bowel of IBS patients is hypersensitive to many different types of stimulation.[9] This information has prompted us to search for a novel 5-HT$_3$ receptor antagonist as a therapeutic agent for the treatment of IBS,[11] as well as for nausea and vomiting associated with cancer chemotherapy.

In our study to find a novel 5-HT$_3$ receptor antagonist possessing more potent activity than **1**, the carbazol-4-one skeleton in **1** was replaced by a thiopyrano[2,3-*b*]indol-4-one skeleton (Chart 1). Initially 9-methyl-3-[(5-methylimidazol-4-yl)methyl]-2,3,4,9-tetrahydrothiopyrano[2,3-*b*]indol-4-one **4c** was found to be a potent 5-HT$_3$ receptor antagonist. Its *exo*-olefin derivative **3c** (an intermediate in the preparation of **4c**) and its *endo*-olefin derivative **2c** (a by-product of the preparation of **3c**) were also found to possess 5-HT$_3$ receptor-antagonistic activity. In this paper, we report the synthesis and structure–activity relationships (SAR) of various thiopyrano[2,3-*b*]indol-4-one derivatives **2b—e**, **3b—e** and **4a—e**.

Chemistry

Synthesis The synthetic methods for **2b—e**, **3b—e** and **4a—e** are shown in Chart 2.

The key intermediate **7** was prepared from 1-methyl-2,3-dihydroindole-2-thione **5**[12] by *S*-alkylation to afford the carboxylic acid **6**, followed by intramolecular Friedel–Crafts acylation. Among the other intermediates **9**, **9b** (R=H)[13] and **9c** (R=Me)[14] were prepared according to the methods described in the cited references. Compound **9d** (R=Et) was obtained as a minor product (10%) of the tritylation of 5-ethylimidazole-4-carboxaldehyde **8**[15] using triethylamine as a base, whereas the isomer **10** was a major product (38%). This regio-

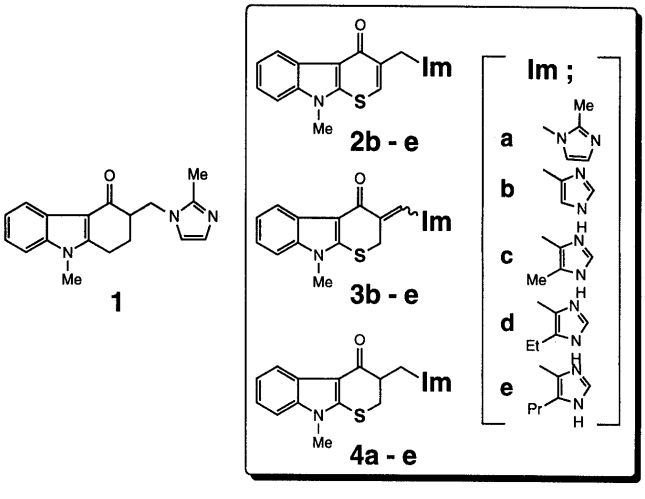


Chart 1

* To whom correspondence should be addressed.

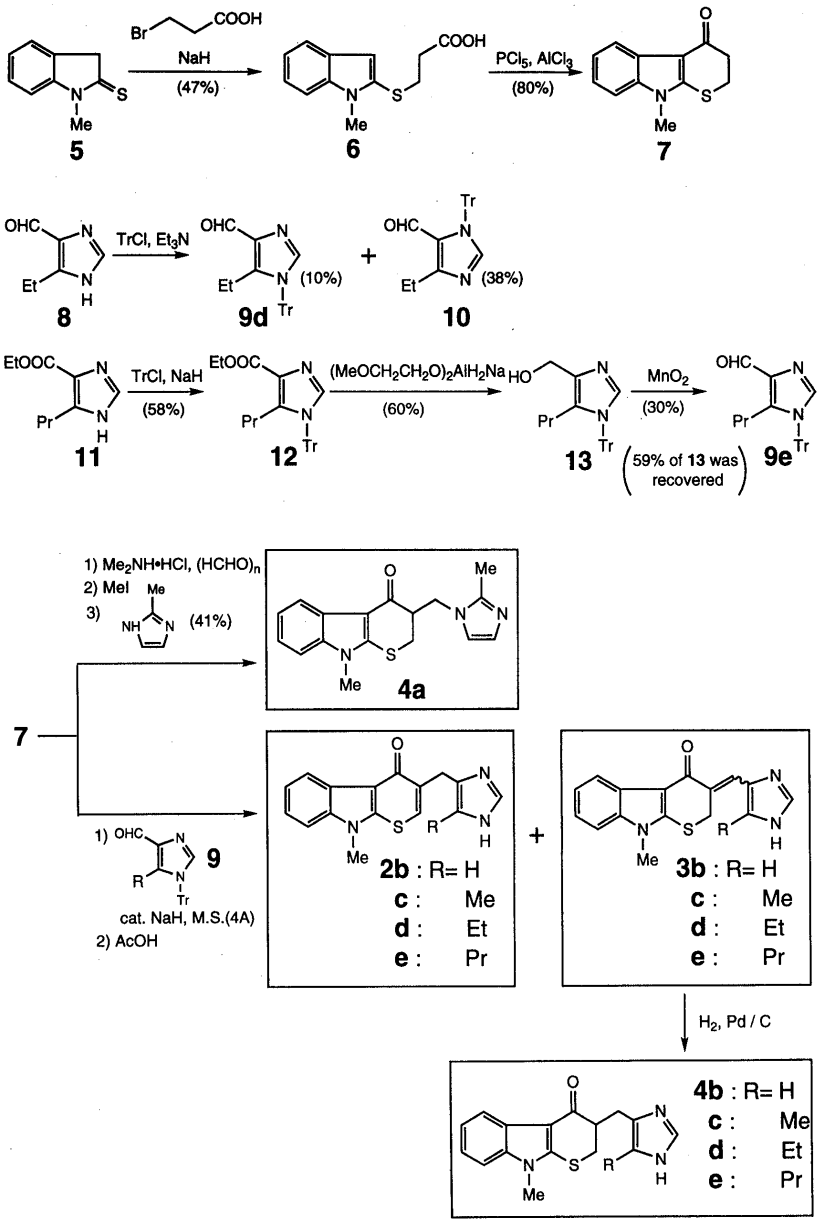


Chart 2

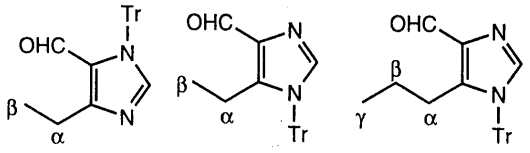
selectivity may be due to the steric effect, since **9b** and **9c** were also obtained as major products from the corresponding aldehydes in a manner similar to that described above for the preparation of **9d**. Therefore, an alternative method was examined to produce compound **9e** (R=Pr) from ethyl 5-propylimidazole-4-carboxylate **11**,[15] as follows. Tritylation of **11**, using sodium hydride instead of triethylamine as a base to generate an anion, proceeded selectively at the 1-position to give the ester **12**. This was converted by reduction with sodium bis(2-methoxyethoxy)aluminum hydride to an alcohol derivative **13**, which was oxidized with manganese(IV) oxide to afford the desired aldehyde derivative **9e**.

The 2-methylimidazol-1-yl derivative **4a** was prepared from **7** by Mannich reaction and quaternization of the dimethylamino group, followed by displacement of the ammonium part by 2-methylimidazole. The condensation reaction of **7** with carboxaldehydes **9** under basic conditions, followed by acid treatment to remove the trityl group, gave a mixture of *endo*-olefin derivatives **2b**—**e** as minor products and *exo*-olefin derivatives **3b**—**e** as major products. This clean and rapid reaction proceeded in the presence of a catalytic amount of sodium hydride in toluene solution, with molecular sieves (M. S., 4A) to remove H_2O. There was a tendency for either a longer reaction time or a larger substituent at the 5-position of imidazole to increase the product ratio of **2b**—**e** relative to **3b**—**e**. Pure **2b**, **2c**, **2e** and **3b**—**e** were obtained by column chromatography and crystallization. However, **2d** was not separated from **3d**. Compounds **4b**—**e** were prepared from the corresponding derivatives **3b**—**e** by catalytic hydrogenation of the *exo*-olefin.

NMR Studies In the ^{1}H-NMR spectrum, upfieldshift of the β- and γ-protons (H^{β} and H^{γ}) in **9e** and β-protons in **9d** were observed (Table 1). In contrast, the formyl proton (H^{CHO}) in **10** appeared at higher field compared with those in **9d** and **9e**.

The structures of **2** and **3** were identified by ^{1}H- and ^{13}C-NMR (heteronuclear multiple bond connectivity; HMBC) studies (Chart 3). Long-range coupling was ob-

Table 1. ^{1}H-NMR Data for **10**, **9d** and **9e** (δ ppm)



	10	**9d**	**9e**
H^{α}	3.00	2.49	2.15—2.55
H^{β}	1.30	0.20	0.25—0.60
H^{γ}	—	—	
H^{CHO}	9.20	9.92	10.05

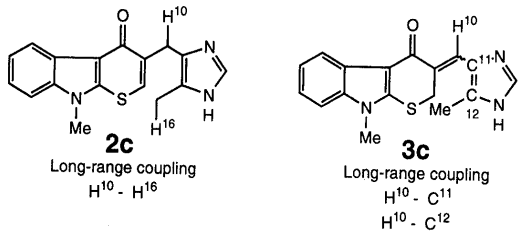
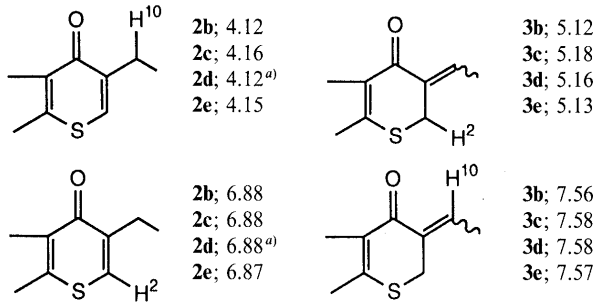


Chart 3

Table 2. ^{1}H-NMR Data for **2** and **3** (δ ppm)



a) In a crude mixture of **2d** and **3d**.

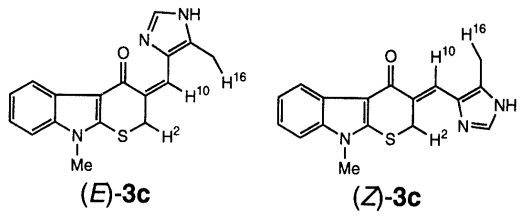


Chart 4

served between H^{10} and H^{16} in **2c** and between H^{10} and each of C^{11} and C^{12} in **3c**. These results suggested that **2c** is the *endo*-olefin derivative and **3c** is the *exo*-olefin isomer. The structure determination of the others was accomplished by comparison of the chemical shifts of each of H^{10} and H^2 (Table 2).

Although each of **3b**—**e** was isolated as a single isomer, the stereochemistry (*E*- or *Z*-) could not be definitively determined. For example, in **3c**, a nuclear Overhauser effect (NOE) was obtained by rotating-frame Overhauser enhancement spectroscopy (ROESY) between H^{10} and each of H^2 or H^{16}, but not between H^2 and H^{16} (Chart 4). These observations might imply the existence of (*E*)-**3c** rather than (*Z*)-**3c**. However, confirmation of the stereochemistry would require comparison of the data for each isomer.

Table 3. 5-HT_3 Receptor-Antagonistic Activities

No.	ID_{50} (μg/kg) of B.J. Reflex[a] [95% confidence limits]	IC_{50} (μM) of colonic contraction[b] [95% confidence limits]
2b	>30	3.8 [1.4—10]
2c	>30	0.28 [0.22—0.35]
2e	>30	0.17 [0.10—0.29]
3b	19 [16—21]	3.8 [3.2—4.6]
3c	8.7 [6.0—13]	0.32 [0.18—0.59]
3d	8.6 [7.5—9.9]	0.33 [0.23—0.46]
3e	>30	0.36 [0.20—0.67]
4a	8.4 [4.6—16]	0.32 [0.18—0.57]
4b	0.26 [0.22—0.31]	0.059 [0.045—0.077]
4c	0.11 [0.070—0.15]	0.0062 [0.0017—0.023]
4d	0.048 [0.026—0.087]	0.030 [0.022—0.042]
4e	0.48 [0.38—0.62]	0.039 [0.024—0.063]
1	3.8 [1.5—7.9]	0.78 [0.43—1.4]

a) i.v., vagally mediated bradycardia induced by 5-HT (10 μg/kg, i.v.) in rats. b) Contraction of isolated guinea pig colon induced by 5-HT (3×10^{-5} M).

Pharmacological Results and Discussion

The 5-HT_3 receptor-antagonistic activities of compounds **2**, **3** and **4** were evaluated by using the 5-HT-induced, vagally mediated bradycardia (von Bezold–Jarisch reflex (B. J. reflex), rats)[16] and the contractile response to 5-HT in the isolated distal colon (guinea pig)[7b] (Table 3). These effects are known to be mediated by activation of the neuronal 5-HT_3 receptors.[7b,16b] Data are presented as the ID_{50} values (μg/kg, i.v.) against the B. J. reflex induced by 5-HT (10 μg/kg, i.v.) in anesthetized rats and IC_{50} values (μM) against the contraction of the isolated guinea pig colon by 5-HT (30 μM).

In spite of the inactivity of the *endo*-olefin derivatives **2b**, **2c** and **2e** in the B.J. reflex test, they were obviously active in the colonic contraction assay; **2c** and **2e** were found to be somewhat more potent (IC_{50} = 0.28 and 0.17 μM, respectively) than **1**. The *exo*-olefin derivatives **3b**, **3c**, **3d**, except **3e**, were shown to be active in the B. J. reflex test, though the potency of each compound was weaker than that of **1**. On the other hand, **3c**, **3d** and **3e** had stronger 5-HT_3 receptor-antagonistic activity than **1** in the colonic contraction assay. Similarly, non-substituted derivatives (**2b**, **3b**) were less potent and the other 5-substituted imidazole derivatives were more potent than **1** in the colonic contraction assay.

Among the saturated compounds **4**, the 2-methyl-1-imidazolyl derivative **4a** possessed similar activities to **1**. The other 5-alkyl-4-imidazolyl derivatives **4b**, **4c**, **4d** and **4e** were found to be strong 5-HT_3 receptor antagonists in both tests. The 5-ethyl derivative **4d** was 79-fold more potent than **1** in the B.J. reflex test, and the 5-methyl derivative **4c** was 126 times more potent than **1** in the colonic contraction assay.

The reduced activity of compounds **2** and **3** compared with **4** may be based on the existence of *endo*- and *exo*-olefin in the 9-methyl-2,3,4,9-tetrahydrothiopyrano-[2,3-*b*]indol-4-one skeleton, while **4** has an sp^3 carbon atom at the 3-position in that skeleton. It is known that

the presence of a basic or quaternary nitrogen atom located in a suitable direction and position relative to the carbonyl oxygen atom is necessary for the antagonists in the binding to the 5-HT_3 receptor.[17] In compounds **3**, the basicity of the imidazole moiety may be lower than that of **4** owing to the *exo*-olefin part, and the location of the basic center might be inadequate. In compounds **2**, the direction and position of the basic center might be unsuitable due to the influence of the *endo*-olefin, despite the existence of the rotatable bond between the imidazole ring and the methylene carbon atom.

Compounds **4** not only have a rotatable bond similar to that in **2**, but also have an additional one between the methylene carbon atom and the sp^3 carbon atom at the 3-position in the 9-methyl-2,3,4,9-tetrahydrothiopyrano-[2,3-*b*]indol-4-one skeleton. It is assumed that both **4c** and **4d** possess the optimum substituent at the 5-position in the imidazole ring to be able to adopt an active conformation for strong 5-HT_3 receptor-antagonistic activity. This might be achieved by restriction of the free rotation between the 5-substituted imidazole and the thiopyrano[2,3-*b*]indole ring because of their steric repulsion.

The C-linked imidazole derivative, in which the 2-methyl-1-imidazolyl group in **1** is replaced by the 5-methyl-4-imidazolyl group, has recently been reported to be more potent than **1**.[18] Our results for **4a** and **4c** are consistent with that report, although the effects of 5-substituents, as in the 5-ethyl and 5-propylimidazole derivatives **4d** and **4e**, have not been reported.

Furthermore, a discrepancy in the SAR was observed in the above two experiments. A similar observation was reported for 4,5,6,7-tetrahydrobenzimidazole derivatives.[17] At present, it is difficult to explain the discrepancy, although the following considerations may apply: (1) species difference or different methods used in *in vivo* or *in vitro* tests could be important; (2) different subtypes of 5-HT_3 receptors might exist in the heart and the colon. These possibilities have also been suggested in other reports.[19] In addition, 9-methyl-2,3,4,9-tetrahydrothiopyrano[2,3-*b*]indol-4-one derivatives **4** were evaluated as their racemates, and the preparation of the optically active isomers is in progress.

Conclusion

Novel 9-methyl-4,9-dihydrothiopyrano[2,3-*b*]indol-4-one derivatives **2b**—**e**, 3-methylene-9-methyl-2,3,4,9-tetrahydrothiopyrano[2,3-*b*]indol-4-one derivatives **3b**—**e** and 9-methyl-2,3,4,9-tetrahydrothiopyrano[2,3-*b*]indol-4-one derivatives **4a**—**e** were synthesized and evaluated for 5-HT_3 receptor-antagonistic activities. Among them, the 5-ethyl-4-imidazolyl derivative **4d** was found to be 79 times more potent than **1** in the B.J. reflex test, and the 5-methyl-4-imidazolyl derivative **4c** was found to be 126 times more potent than **1** in the colonic contraction test. An SAR study suggested that the great potency of **4c** and **4d** could be attributable to a suitable position and direction of the N–C–N centroid in the imidazole ring relative to the carbonyl oxygen atom in binding to the 5-HT_3 receptor. This might be a consequence of restriction of the free rotation between the 5-substituted imidazole and the thiopyrano[2,3-*b*]indole ring because of their steric repulsion.

Experimental

All melting points were determined on a Yanaco MP-500D melting point apparatus and are uncorrected. ^{1}H-NMR spectra were measured with a JEOL FX90Q, FX100, FX270 or FX400 spectrometer; chemical shifts are recorded in δ units using tetramethylsilane as an internal standard, and the following abbreviations are used: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = double doublets, dt = double triplets and tq = triple quartets. Mass spectra were recorded with a Hitachi M-80 (EI), a JEOL JMS-DX300 (FAB) or a VG ZAB-VSE (FAB-HR) spectrometer. Elemental analyses were performed with a Yanaco MT-5. All reactions were carried out in a stream of argon or under an argon atmosphere. All organic solvent extracts were dried over anhydrous magnesium sulfate and concentrated with a rotary evaporator under reduced pressure.

3-[(1-Methylindol-2-yl)thio]propionic Acid (6) 1-Methyl-2,3-dihydroindole-2-thione (**5**,[12] 40.8 g, 250 mmol) was added portionwise to a suspension of sodium hydride (20.0 g, 500 mmol; 60%) in dimethylformamide (DMF) (375 ml) over 20 min below 5 °C. The mixture was stirred for 30 min, then 3-bromopropionic acid (38.2 g, 250 mmol) was added portionwise. The whole was allowed to warm to room temperature over 1 h, stirred overnight and concentrated. The residure was taken up in H_2O (250 ml). The mixture was washed with Et_2O, acidified (pH *ca.* 2—3) by the addition of concentrated HCl, and extracted with $CHCl_3$. The organic solution was dried and concentrated. The residue was purified by silica gel column chromatography ($CHCl_3$–MeOH) and washed with iso-Pr_2O to give 46.5 g (79% yield) of **6** as a solid; ^{1}H-NMR ($CDCl_3$) δ: 2.56 (2H, t, J=7.8 Hz), 2.98 (2H, t, J=7.8 Hz), 3.76 (3H, s), 6.70 (1H, d, J=1.4 Hz), 6.90—7.24 (2H, m), 7.30—7.60 (2H, m). HR-FAB-MS *m/z*: Calcd for $C_{12}H_{14}NO_2S$ $(M+H)^+$: 236.0745. Found: 236.0753.

9-Methyl-2,3,4,9-tetrahydrothiopyrano[2,3-*b*]indol-4-one (7) PCl_5 (20.9 g, 100 mmol) was added portionwise to a solution of carboxylic acid **6** (21.5 g, 91 mmol) in CH_2Cl_2 (215 ml) over 10 min at room temperature. The reaction was exothermic and yielded a white precipitate. The mixture was stirred for 1 h, then $AlCl_3$ (14.6 g, 109 mmol) was added portionwise below 25 °C. The resulting mixture was stirred for 90 min, poured into ice-cold water and extracted with $CHCl_3$ (200 and 100 ml). The combined organic layer was washed with dilute HCl, H_2O and saturated aqueous $NaHCO_3$, dried, concentrated and washed with iso-Pr_2O to afford 15.9 g (80% yield) of **7** as a solid; ^{1}H-NMR ($CDCl_3$) δ: 2.70—3.00 (2H, m), 3.20—3.55 (2H, m), 3.65 (3H, s), 7.10—7.35 (3H, m), 8.15—8.40 (1H, m). *Anal.* Calcd for $C_{12}H_{11}NOS$: C, 66.33; H, 5.10; N, 6.45; S, 14.76. Found: C, 66.26; H, 5.03; N, 6.41; S, 14.63. HR-FAB-MS *m/z*: Calcd for $C_{12}H_{12}NOS$ $(M+H)^+$: 218.0640. Found: 218.0642.

5-Ethyl-1-tritylimidazole-4-carboxaldehyde (9d) and 4-Ethyl-1-tritylimidazole-5-carboxaldehyde (10) A solution of trityl chloride (7.81 g, 28.4 mmol) in CH_2Cl_2 (25 ml) was added dropwise to a suspension of 5-ethylimidazole-4-carboxaldehyde (**8**,[15] 3.55 g, 28.4 mmol) and triethylamine (2.8 g, 28.4 mmol) in CH_2Cl_2 (60 ml) at room temperature, and the mixture was stirred overnight, washed with H_2O, dried and concentrated. The residue was purified by silica gel column chromatography ($CHCl_3$) to give 1.0 g (10% yield) of **9d** and 4.0 g (38% yield) of **10**, each as a powder. For **9d**; ^{1}H-NMR ($CDCl_3$) δ: 0.20 (3H, t, J=7 Hz), 2.49 (2H, q, J=7 Hz), 7.00—7.50 (16H, m), 9.92 (1H, s). HR-FAB-MS *m/z*: Calcd for $C_{25}H_{23}N_2O$ $(M+H)^+$: 367.1810. Found: 367.1819. For **10**; ^{1}H-NMR ($CDCl_3$) δ: 1.30 (3H, t, J=7 Hz), 3.00 (2H, q, J=7 Hz), 7.00—7.50 (16H, m), 9.20 (1H, s). EI-MS *m/z*: 367 (M^+).

Ethyl 5-Propyl-1-tritylimidazole-4-carboxylate (12) Sodium hydride (3.46 g, 86.5 mmol; 60%) was added portionwise over 30 min to a solution of ethyl 5-propylimidazole-4-carboxylate (**11**,[15] 13.12 g, 72.0 mmol) in tetrahydrofuran (THF) (100 ml) below 5 °C. Trityl chloride (23.1 g, 82.9 mmol) was added portionwise to the suspension. The mixture was allowed to warm to room temperature over 1 h, heated overnight at 35 °C, poured into cold 1 N HCl (100 ml), neutralized by addition of $NaHCO_3$, dried and concentrated. The residue was purified by silica gel column chromatography ($CHCl_3$–MeOH) to give 17.70 g (58% yield) of **12** as a foam; ^{1}H-NMR ($CDCl_3$) δ: 0.20—0.50 (5H, m), 1.38 (3H, t, J=7.2 Hz), 2.30—2.50 (2H, m), 4.35 (2H, q, J=7.2 Hz), 7.00—7.40 (16H, m). HR-FAB-MS *m/z*: Calcd for $C_{28}H_{29}N_2O_2$ $(M+H)^+$: 425.2229.

Found: 425.2226.

4-Hydroxymethyl-5-propyl-1-tritylimidazole (13) A solution of sodium bis(2-methoxyethoxy)aluminum hydride in toluene (3.4 M; 28.2 ml, 96 mmol) was added dropwise over 2 h to a solution of **12** (16.6 g, 39.1 mmol) in toluene (165 ml) below 30 °C. The mixture was stirred overnight at room temperature, then 1 N NaOH (300 ml) was added dropwise below 10 °C. The organic layer was separated and the aqueous layer was extracted with toluene (150 ml), twice. Brine was added to the aqueous layer and the mixture was extracted with CH_2Cl_2–MeOH. The combined organic layer was dried, concentrated and washed with iso-Pr_2O to give 9.01 g (60% yield) of **13** as a solid; ^{1}H-NMR ($CDCl_3$) δ: 0.20—0.70 (5H, m), 1.90—2.40 (2H, m), 4.55 (2H, s), 7.00—7.50 (16H, m). HR-FAB-MS *m/z*: Calcd for $C_{26}H_{27}N_2O$ $(M+H)^+$: 383.2123. Found: 383.2132.

5-Propyl-1-tritylimidazole-4-carboxaldehyde (9e) A mixture of **13** (8.31 g, 21.7 mmol) and manganese(IV) oxide (19.4 g, 228 mmol) in dioxane (330 ml) was heated overnight at 85 °C. The insoluble material was filtered off, and the filtrate was concentrated. The residue was purified by silica gel column chromatography ($CHCl_3$–MeOH) to yield recovered **13** (4.89 g, 59% yield) and 2.47 g (30% yield) of **9e** as a solid; ^{1}H-NMR ($CDCl_3$) δ: 0.25—0.60 (5H, m), 2.15—2.55 (2H, m), 7.00—7.50 (16H, m), 10.05 (1H, s). HR-FAB-MS *m/z*: Calcd for $C_{26}H_{25}N_2O$ $(M+H)^+$: 381.1967. Found: 381.1962.

9-Methyl-3-[(2-methylimidazol-1-yl)methyl]-2,3,4,9-tetrahydrothiopyrano[2,3-*b*]indol-4-one (4a) (i) A mixture of **7** (0.42 g, 1.93 mmol), dimethylamine hydrochloride (0.24 g, 2.9 mmol) and paraformaldehyde (0.10 g, 3.3 mmol) in MeOH (2 ml) was heated under reflux for 20 h. A precipitate was collected by filtration and poured into a mixture of $CHCl_3$ and saturated aqueous $NaHCO_3$. The organic layer was dried and concentrated *in vacuo* to give 0.30 g of crude 9-methyl-3-(dimethylaminomethyl)-2,3,4,9-tetrahydrothiopyrano[2,3-*b*]indol-4-one as a foam; ^{1}H-NMR ($CDCl_3$) δ: 2.32 (6H, s), 2.50—3.00 (3H, m), 3.40—3.65 (2H, m), 3.70 (3H, s), 7.15—7.35 (3H, m), 8.15—8.30 (1H, m). EI-MS *m/z*: 274 (M^+).

(ii) Methyl iodide (0.08 ml, 1.3 mmol) was added to a solution of the crude 9-methyl-3-(dimethylaminomethyl)-2,3,4,9-tetrahydrothiopyrano-[2,3-*b*]indol-4-one (0.30 g) in DMF (5 ml). The mixture was stirred for 30 min at room temperature, then 2-methylimidazole (0.42 g, 5.1 mmol) was added. The whole was heated at 80 °C for 4 h, $CHCl_3$ (15 ml) was added to it, and the organic layer was washed with H_2O, dried and concentrated. The residue was purified by silica gel column chromatography ($CHCl_3$–MeOH) to give 0.25 g (41% yield from 7) of **4a** as a solid; mp 207—209 °C (from EtOH–Et_2O). ^{1}H-NMR ($CDCl_3$) δ: 2.46 (3H, s), 3.00—3.40 (3H, m), 3.72 (3H, s), 4.15 (1H, dd, J=9.0, 14.0 Hz), 4.50 (1H, dd, J=9.0, 14.0 Hz), 6.94 (1H, d, J=2.0 Hz), 6.98 (1H, d, J=2.0 Hz), 7.20—7.40 (3H, m), 8.20—8.40 (1H, m). EI-MS *m/z*: 311 (M^+). *Anal.* Calcd for $C_{17}H_{17}N_3OS \cdot 0.3H_2O$: C, 64.45; H, 5.60; N, 13.26; S, 10.12. Found: C, 64.51; H, 5.43; N, 13.22; S, 10.33.

9-Methyl-3-[(5-methylimidazol-4-yl)methyl]-4,9-dihydrothiopyrano-[2,3-*b*]indol-4-one (2c) and 9-Methyl-3-[(5-methylimidazol-4-yl)methylene]-2,3,4,9-tetrahydrothiopyrano[2,3-*b*]indol-4-one (3c) A mixture of **7** (53.8 g, 248 mmol), 5-methyl-1-tritylimidazole-4-carboxaldehyde (**9c**)[14] solvated with dioxane (141.7 g, 322 mmol), sodium hydride (1.98 g, 50 mmol; 60%) and molecular sieves (4A, 160 g) in toluene (1100 ml) was heated under reflux for 2 h with azeotropic removal of H_2O. After the mixture had cooled to room temperature, AcOH (6.5 ml) was added to it. An insoluble material was filtered off, and the filtrate was concentrated. A mixture of THF (540 ml), AcOH (540 ml) and H_2O (540 ml) was added to the residue, and the mixture was heated under reflux for 1.5 h and concentrated. A mixture of a saturated aqueous $NaHCO_3$ and $CHCl_3$ was added to the residue, and the organic layer was washed with brine, dried, and concentrated. The residue was crystallized from AcOEt to give 47.96 g (63% yield) of **3c** as a solid; mp 195—202 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.42 (3H, s), 3.72 (3H, s), 5.00 (2H, s), 7.20—7.40 (3H, m), 7.58 (1H, s), 8.20—8.40 (1H, m). EI-MS *m/z*: 309 (M^+). *Anal.* Calcd for $C_{17}H_{15}N_3OS \cdot H_2O$: C, 62.37; H, 5.23; N, 12.83; S, 9.79. Found: C, 62.75; H, 4.89; N, 12.70; S, 9.75. The filtrate was concentrated, and the residue was purified by silica gel column chromatography ($CHCl_3$–MeOH) to give another 4.56 g (6% yield) of **3c** and 5.3 g (7% yield) of **2c** as a solid; mp 217—219 °C (crystallized from AcOEt). ^{1}H-NMR ($CDCl_3$) δ: 2.42 (3H, s), 3.76 (3H, s), 4.18 (2H, s), 6.88 (1H, s), 7.20—7.40 (3H, m), 8.30—8.50 (1H, m). EI-MS *m/z*: 309 (M^+). *Anal.* Calcd for $C_{17}H_{15}N_3OS \cdot 0.11H_2O$: C, 65.57; H, 4.93; N, 13.50; S, 10.30. Found: C, 65.56; H, 4.82; N, 13.36; S, 10.59.

9-Methyl-3-[(imidazol-4-yl)methyl]-4,9-dihydrothiopyrano[2,3-*b*]indol-4-one (2b) and 9-Methyl-3-[(imidazol-4-yl)methylene]-2,3,4,9-tetrahydrothiopyrano[2,3-*b*]indol-4-one (3b) **2b** and **3b** were prepared in 3 and 31% yields, respectively, in a similar manner to that described for **2c** and **3c**, using 1-tritylimidazole-4-carboxaldehyde (**9b**)[13] instead of **9c**. For **2b**; mp 184—190 °C (crystallized from MeOH–iso-Pr_2O). ^{1}H-NMR ($CDCl_3$) δ: 3.68 (3H, s), 4.12 (2H, s), 6.88 (1H, s), 7.20—7.40 (4H, m), 7.68 (1H, s), 8.30—8.40 (1H, m). HR-FAB-MS *m/z*: Calcd for $C_{16}H_{14}N_3OS$ $(M+H)^+$: 296.0858. Found: 296.0865. For **3b**; mp 218—221 °C (crystallized from MeOH–iso-Pr_2O). ^{1}H-NMR ($CDCl_3$) δ: 3.72 (3H, s), 5.12 (2H, s), 7.20—7.40 (4H, m), 7.56 (1H, s), 7.68 (1H, s), 8.20—8.40 (1H, m). FAB-MS *m/z*: 296 ($M^+ + 1$). *Anal.* Calcd for $C_{16}H_{13}N_3OS$: C, 65.06; H, 4.44; N, 14.23; S, 10.86. Found: C, 64.76; H, 4.50; N, 14.06; S, 11.03.

9-Methyl-3-[(5-ethylimidazol-4-yl)methylene]-2,3,4,9-tetrahydrothiopyrano[2,3-*b*]indol-4-one (3d) **3d** was prepared in 24% yield in a similar manner to that described for **2c** and **3c**, using **9d** instead of **9c**; mp 185—188 °C (crystallized from AcOEt). ^{1}H-NMR ($CDCl_3$) δ: 1.24 (3H, t, J=7.0 Hz), 1.25 (0.3H, t, J=6.5 Hz, for 0.1 AcOEt), 2.05 (0.3H, s, for 0.1 AcOEt), 2.80 (2H, q, J=7.0 Hz), 3.68 (3H, s), 4.12 (0.2 H, q, J=6.5 Hz, for 0.1 AcOEt), 5.16 (2H, s), 7.20—7.40 (4H,m), 7.58 (1H, s), 8.20—8.40 (1H, m). EI-MS *m/z*: 323 (M^+). *Anal.* Calcd for $C_{18}H_{17}N_3OS \cdot 0.1H_2O \cdot 0.1$AcOEt: C, 66.16; H, 5.43; N, 12.58; S, 9.60. Found: C, 66.16; H, 5.36; N, 12.33; S, 9.85.

9-Methyl-3-[(5-propylimidazol-4-yl)methyl]-4,9-dihydrothiopyrano-[2,3-*b*]indol-4-one (2e) and 9-Methyl-3-[(5-propylimidazol-4-yl)methylene]-2,3,4,9-tetrahydrothiopyrano[2,3-*b*]indol-4-one (3e) **2e** and **3e** were prepared in 22 and 48% yields, respectively, in a similar manner to that described for **2c** and **3c**, using **9e** instead of **9c**. For **2e**; mp 198—202 °C (as a HCl salt from EtOH–AcOEt). ^{1}H-NMR ($CDCl_3$) δ: 0.98 (3H, t, J=7.5 Hz), 1.74 (2H, dt, J= 7.5, 7.5 Hz), 2.70 (2H, t, J=7.5 Hz), 3.70 (3H, s), 4.15 (2H, s), 6.87 (1H, s), 7.25—7.40 (3H, m), 7.61 (1H, s), 8.30—8.45 (1H, m). EI-MS *m/z*: 337 (M^+). *Anal.* Calcd for $C_{19}H_{19}N_3OS \cdot$HCl: C, 61.03; H, 5.39; N, 11.24; S, 8.58; Cl, 9.48. Found: C, 60.72; H, 5.38; N, 10.88; S, 8.45; Cl, 9.74. For **3e**; mp 193—196 °C (as a HCl salt from EtOH–AcOEt). ^{1}H-NMR ($CDCl_3$) δ: 0.86 (3H, t, J=7.5 Hz), 1.60 (2H, dt, J=7.5 Hz), 2.64 (2H, t, J=7.5 Hz), 3.67 (3H, s), 5.13 (2H, s), 7.20—7.35 (3H, m), 7.5 (1H, s), 7.62 (1H, s), 8.25—8.35 (1H, m). EI-MS *m/z*: 337 (M^+). As a HCl salt; ^{1}H-NMR (DMSO-d_6) δ: 0.86 (3H, t, J=7.5 Hz), 1.06 (1.2H, t, J=6.5 Hz, for 0.4 EtOH), 1.66 (2H, tq, J= 7.5, 7.5 Hz), 2.70 (2H, t, J=7.5 Hz), 3.44 (0.8H, q, J=6.5 Hz, for 0.4 EtOH), 3.62 (3H, s), 4.48 (2H, s), 7.16—7.36 (3H, m), 7.42—7.60 (1H, m), 8.00—8.16 (1H, m), 9.14 (1H, s). *Anal.* Calcd for $C_{19}H_{19}N_3OS \cdot$HCl$\cdot 0.4$EtOH: C, 60.62; H, 5.75; N, 10.71; S, 8.17; Cl, 9.04. Found: C, 60.27; H, 5.49; N, 10.67; S, 8.31; Cl, 9.44.

9-Methyl-3-[(5-methylimidazol-4-yl)methyl]-2,3,4,9-tetrahydrothiopyrano[2,3-*b*]indol-4-one Fumarate (4c) A solution of **3c** (50.0 g, 162 mmol) in MeOH (1.5 l) and concentrated HCl (16 ml) was hydrogenated over 10% palladium-on-carbon (50% wet, 50 g) at 40 °C. Insoluble material was filtered off, and the filtrate was concentrated. A mixture of saturated aqueous $NaHCO_3$ and $CHCl_3$ was added to the residue, and the organic layer was washed with brine, dried, and concentrated. The residue was purified by silica gel column chromatography ($CHCl_3$–MeOH) to give a foam, which was treated with one equivalent of fumaric acid in MeOH to afford 35.8 g (52% yield) of **4c** as a solid; mp 195—202 °C (from MeOH). ^{1}H-NMR ($CDCl_3$) δ: 2.12 (3H, s), 2.60—3.60 (5H, m), 3.72 (3H, s), 6.60 (2H, s), 7.10—7.30 (2H, m), 7.40—7.60 (1H, m), 7.62 (1H, s), 8.00—8.10 (1H, m). EI-MS *m/z*: 311 (M^+). *Anal.* Calcd for $C_{17}H_{17}N_3OS \cdot C_4H_4O_4$: C, 57.31; H, 5.13; N, 9.55; S, 7.29. Found: C, 57.25; H, 4.94; N, 9.47; S, 7.10.

9-Methyl-3-[(imidazol-4-yl)methyl]-2,3,4,9-tetrahydrothiopyrano[2,3-*b*]indol-4-one (4b) **4b** was prepared in 15% yield in a similar manner to that described for **4c**; mp 75—79 °C (from EtOH–AcOEt). ^{1}H-NMR ($CDCl_3$) δ: 1.25 (1.5H, t, J=6.5 Hz, for 0.5 AcOEt), 2.05 (1.5H, s, for 0.5 AcOEt), 3.00—3.40 (5H, m), 3.66 (3H, s), 4.12 (1H, q, J=6.5 Hz, for 0.5 AcOEt), 6.88 (1H, s), 7.20—7.40 (3H, m), 7.56 (1H, s), 8.20—8.40 (1H, m). FAB-MS *m/z*: 298 ($M^+ + 1$). *Anal.* Calcd for $C_{16}H_{15}N_3OS \cdot 0.6H_2O \cdot 0.5$AcOEt: C, 61.38; H, 5.78; N, 11.93; S, 9.10. Found: C, 61.27; H, 5.45; N, 12.15; S, 8.81.

9-Methyl-3-[(5-ethylimidazol-4-yl)methyl]-2,3,4,9-tetrahydrothiopyrano[2,3-*b*]indol-4-one (4d) **4d** was prepared in 23% yield in a similar manner to that described for **4c**; mp 233—237 °C (EtOH–AcOEt). ^{1}H-NMR ($CDCl_3$) δ: 1.24 (3H, t, J=7.0 Hz), 2.60 (2H, t, J=7.0 Hz), 3.00—3.40 (5H, m), 3.68 (3H, s), 7.20—7.40 (3H, m), 7.48 (1H, s),

8.20—8.40 (1H, m). EI-MS *m/z*: 325 (M^+). *Anal.* Calcd for $C_{18}H_{19}N_3OS$: C, 66.07; H, 5.91; N, 12.84; S, 9.80. Found: C, 65.82; H, 5.97; N, 12.64; S, 9.74.

9-Methyl-3-[(5-propylimidazol-4-yl)methyl]-2,3,4,9-tetrahydrothiopyrano[2,3-*b*]indol-4-one Fumarate (4e) **4e** was prepared in 52% yield in a similar manner to that described for **4c**; mp 171—173 °C (MeOH–iso-Pr_2O). ^{1}H-NMR (DMSO-d_6) d: 0.86 (3H, t, J=7.5 Hz), 1.62 (2H, dt, J=7.5 Hz), 2.68 (2H, t, J=7.5 Hz), 2.60—2.80 (1H, m), 2.80—3.00 (1H, m), 3.00—3.12 (1H, m), 3.12—3.28 (1H, m), 3.40—3.56 (1H, m), 3.72 (3H, s), 6.64 (2H, s), 7.16—7.30 (2H, m), 7.48—7.54 (1H, s), 7.76 (1H, s), 8.04—8.12 (1H, m). EI-MS *m/z*: 339 (M^+). *Anal.* Calcd for $C_{19}H_{21}N_3OS \cdot C_4H_4O_4 \cdot 0.3H_2O$: C, 59.93; H, 5.60; N, 9.12; S, 6.96. Found: C, 59.86; H, 5.50; N, 9.04; S, 7.11.

Biological Methods Doses are expressed in terms of free base. 5-HT was purchased from E. Merck (Darmstadt, FRG) as creatinine sulfate.

B.J. Reflex Test[16b] Male Wistar rats weighing 200 to 250 g were anesthetized with urethane (1.25 g/kg i.p.), and the trachea was cannulated. Arterial blood pressure and heart rate were recorded on a polygraph through a pressure transducer and a cardiotachometer, respectively, connected to a catheter placed in the carotid artery. The femoral vein was also cannulated for drug injection. 5-HT at a dose of 10 μg/kg was intravenously administered to rats at intervals of 15 min. After a stable response to 5-HT had been obtained, drugs were intravenously administered to rats 10 min before 5-HT injection.

Contraction of Isolated Guinea Pig Colon[7b] The distal portion of the colon was removed from Hartley guinea pigs (300 to 500 g), cleaned in fresh Krebs-bicarbonate buffer at room temperature and then divided into approximately 20 mm segments. Isometric contraction under a loading tension of 1 g was recorded. Submaximal contraction was first elicited by repeated applications of 10^{-6} M 5-HT until a constant response was obtained. Test compounds were added to the bath after a concentration-response curve for 5-HT had been obtained. The tissue was exposed to the test compound for 30 min before rechallenge with 5-HT (control). Each test compound was examined at one or two different concentrations in the same preparation.

Acknowledgments We thank Drs. N. Inukai and M. Takeda for their support during the course of this work, Dr. K. Murase for his valuable suggestions and encouragement, Drs. T. Mase and Y. Katsuyama for their helpful discussions, and Messrs H. Kaniwa, M. Shimizu, and the staff of the Structure Analysis Department for spectral measurements and elemental analyses.

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