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Novel Antiasthmatic Agents with Dual Activities of Thromboxane A_2 Synthetase Inhibition and Bronchodilation. V.[1]

Thienopyridazinone Derivatives

Masahisa YAMAGUCHI,* Noriaki MARUYAMA, Takaki KOGA, Kenshi KAMEI,
Michitaka AKIMA, Toshio KUROKI, Masatomo HAMANA, and Nobuhiro OHI

Fuji-gotemba Research Laboratories, Chugai Pharmaceutical Company, Ltd., 135, 1-Chome Komakado, Gotemba-shi, Shizuoka, 412 Japan.
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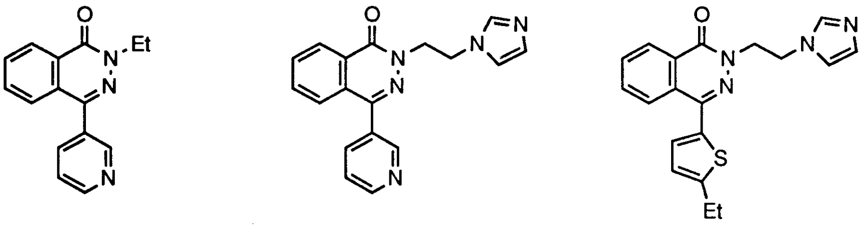
Synthesis and pharmacological evaluation of novel thienopyridazinones and related compounds are described. A thiophene ring was found to be able to replace the benzene ring of a phthalazinone without loss of biological activities. This observation supports our hypothesis that the benzene ring plays an important role in both thromboxane A_2 (TXA_2) synthetase-inhibitory and bronchodilatory activities. Further, it was shown that the carbonyl moiety of a phthalazinone is not necessary for these activities.

Key words thienopyridazinone; TXA_2 synthetase inhibitor; bronchodilator; antiasthmatic agent

We have previously reported that 2,4-disubstituted 1(2*H*)-phthalazinone derivatives, such as 2-ethyl-4-(3-pyridyl)- (**1**),[2] 2-[2-(1-imidazolyl)ethyl]-4-(3-pyridyl)-(**2**),[3] and 4-[2-(5-ethylthienyl)]-2-[2-(1-imidazolyl)ethyl]-1(2*H*)-phthalazinone (**3**).[3] have thromboxane A_2 (TXA_2) synthetase-inhibitory and bronchodilating activities. Among these compounds, **1** showed the most satisfactory and well-balanced potencies. We have examined the structure–activity relationship of **1** in detail and found that the 3-pyridyl group at the 4-position is an essential component for both activities and that an ethyl group is the most effective among the tested 2-substituents.[2] Based on the study with compound **2**, the benzene ring of the phthalazinone skeleton was also demonstrated to play an important role in both activities; either removal of the benzene ring or introduction of a substituent into the benzene ring led to a significant reduction of both activities.[1] Modification of the benzene ring of compound **3** gave similar results.[4] Because a thiophene ring is, in general, considered to be a bioisostere of a benzene ring, these observations led us to synthesize thienopyridazinone derivatives of **1** and to examine their activities in order to clarify further the role of the phenyl moiety of the phthalazinone skeleton in both activities. In addition, we prepared furopyridazinone as a structurally related compound to compare its activities with those of the thienopyridazinone derivatives. Further, in order to examine the role of the carbonyl group at the 1-position of the phthalazinone, which has not been addressed previously, we synthesized a 1-ethoxy-thienopyridazine derivative and evaluated its pharmacological activities.

Thienopyridazinone derivatives (**7** and **12a**) were prepared from 3-(3-pyridinoyl)thiophene-2-carboxylic acid (**5**) and 2-(3-pyridinoyl)thiophene-3-carboxylic acid (**10a**), respectively, in the same way as described in a previous paper (Chart 1).[2] Preparation of **5** was performed by sequential *n*-butyllithium (*n*-BuLi)-mediated reactions of 3-bromothiophene (**4**) in a one-pot procedure according to the reported method.[5] Thus, treatment of **4** with one equivalent of *n*-BuLi and 3-cyanopyridine followed by treatment with another equivalent of *n*-BuLi and dry ice, and hydrolysis with diluted hydrochloric acid gave **5** in a crude form. Cyclization of the mixture with hydrazine hydrate gave **6** in a low yield of 7% from **4**. In a similar manner, **11a** was obtained from 2,3-dibromothiophene (**8**) without isolation of **10a** again in a low yield of 10%. Transformation of **6** and **11a** into the desired **7** and **12a**, respectively, was achieved by ethylation (Chart 1).

Because the above syntheses of **5** and **10a** through lithiation of bromothiophene were quite unsatisfactory with respect to yield, we tried the application of the dianion[6] derived from easily available thiophene-3-carboxylic acid (**9a**) to the preparation of **10a**. The dianion formed from **9a** by means of two equivalents of lithium diisopropylamide was treated with 3-pyridinecarbaldehyde to give 2-(3-pyridyl)hydroxymethylthiophene-3-carboxylic



1 **2** **3**

Fig. 1. 2-Ethyl-4-(3-pyridyl)-(**1**), 2-[2-(1-Imidazolyl)ethyl]-4-(3-pyridyl)-(**2**) and 4-[2-(5-Ethylthienyl)]-2-[2-(1-imidazolyl)ethyl]-1(2*H*)-phthalazinone (**3**)

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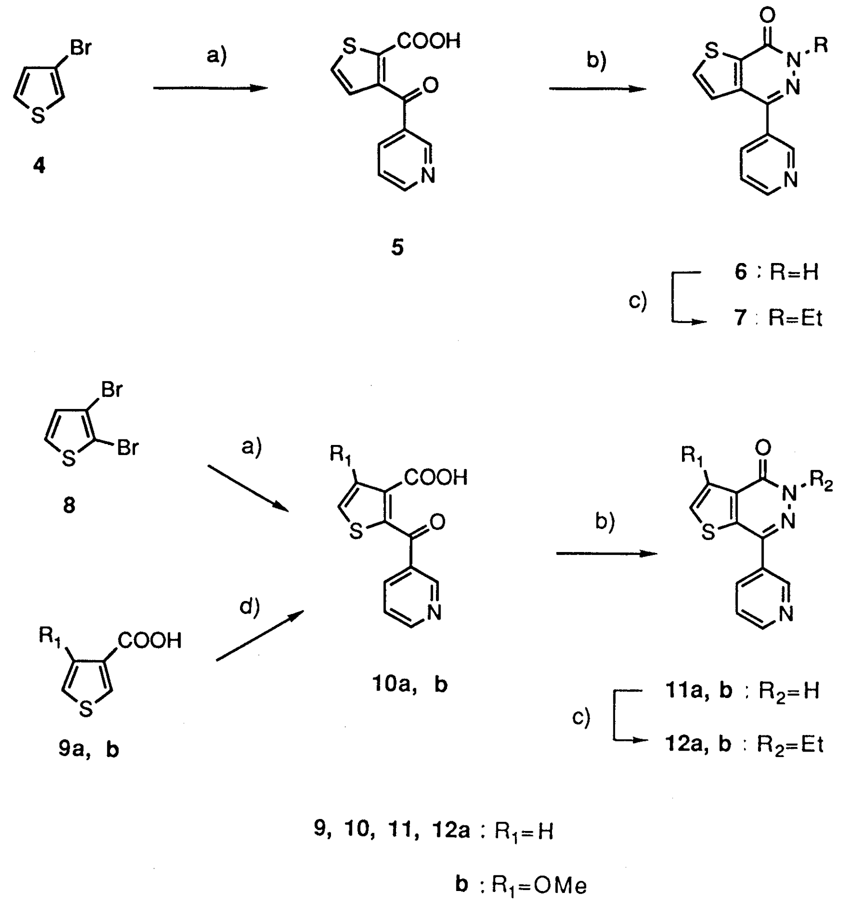
* To whom correspondence should be addressed.



a) (i) *n*-BuLi, −78 °C, 3-cyanopyridine; (ii) *n*-BuLi, −5 °C, dry ice; (iii) HCl aq.; b) H_2NNH_2, EtOH, reflux; c) EtBr, NaH; d) (i) 2 eq of LDA, −78 °C, 3-pyridinecarbaldehyde; (ii) $KMnO_4$.

Chart 1

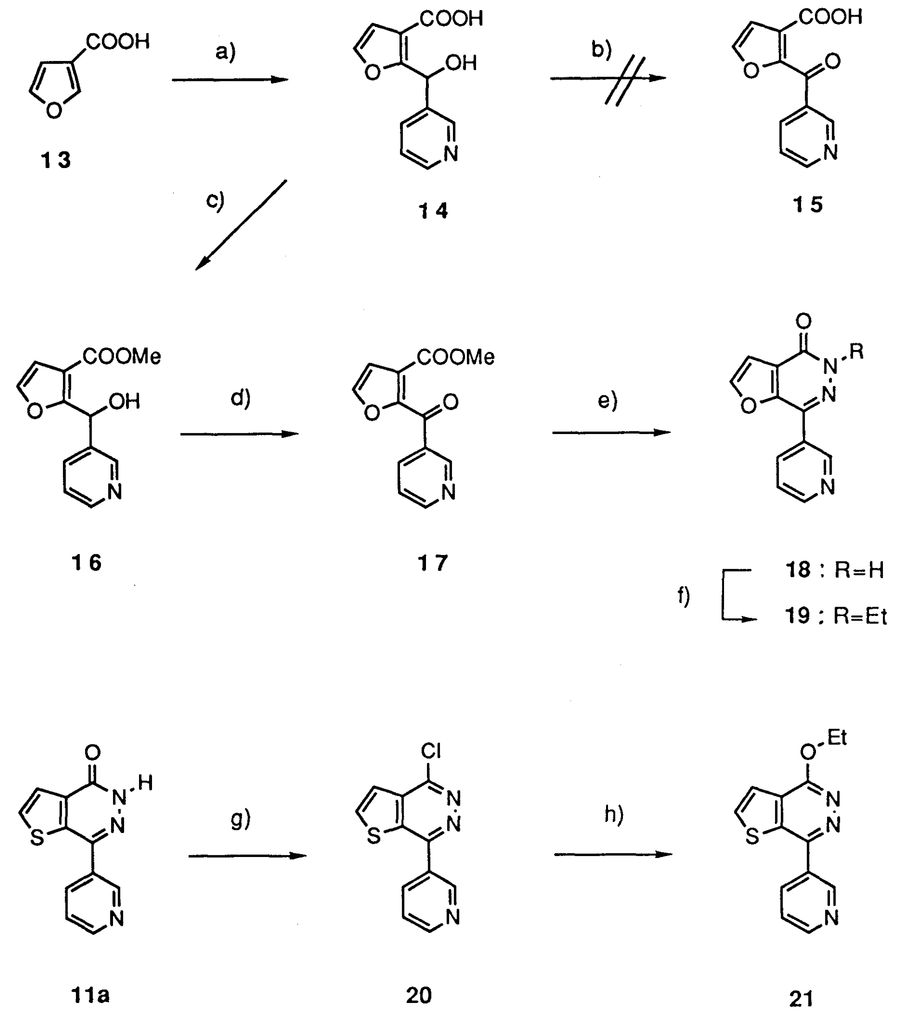
acid, which was easily oxidized with potassium permanganate to afford **10a** in 65% yield. By the same procedure, the 4-methoxy derivative of **10a** (**10b**) was also prepared in 58% yield from 4-methoxythiophene-3-carboxylic acid (**9b**) (Chart 1).

Subsequently we applied this method to the synthesis of 2-(3-pyridinoyl)furan-3-carboxylic acid (**15**) from furan-3-carboxylic acid (**13**). Although the initial carbinol product (**14**) was formed in the same way, **14** resisted oxidation with potassium permanganate and did not give the desired **15**. Then, **14** was converted to the methyl ester (**16**) which was subjected to Swern oxidation[7] using oxalyl chloride to give methyl 2-(3-pyridinoyl)thiophene-3-carboxylate (**17**) in 78% overall yield from **13**. Cyclization of **17** with hydrazine hydrate to the furopyridazinone (**18**), followed by ethylation gave the furopyridazinone analogue of **12a** (**19**) (Chart 2).

4-Ethoxy-7-(3-pyridyl)-thieno[2,3-*d*]pyridazine (**21**) was readily prepared from the corresponding thienopyridazinone (**11a**) in 70% yield *via* a 4-chloro compound (**20**) in the usual manner (Chart 2).

TXA_2 synthetase-inhibitory and bronchodilatory activities were examined by using the previously described assay systems.[2] Thienopyridazinone derivatives without substitution on the thiophene moiety (**7** and **12a**) exhibited high and well-balanced activities according to our expectation. In particular, **12a** was shown to be as active as the parent compound **1** (Table I). On the other hand, **12b** bearing a methoxy group on the thiophene ring showed weaker activities. Further, the furopyridazinone derivative **19** exhibited must weaker bronchodilatory activity (Table I). These results in this limited study indicate that a thiophene ring can replace the benzene ring, but a furan ring cannot. Further, although only the methoxy group was introduced because of the difficulty of the synthesis, it was suggested that introduction of a substituent into the mother skeleton reduces both activities. These observations are consistent with our previous findings[1,4] on **2** snd **3**. Reduction of the activities of the furopyridazinone (**19**) may be due to the increase in hydrophilicity as well. Of particular interest is the finding that **21** exhibited almost the same potency as **12a**. This observation demonstrates that the carbonyl moiety of thienopyridazinone is not necessary for the activities, which is an important result concerning the structure–activity relationship of phthalazinone derivatives.

In conclusion, we have found that the thienopyridazinone derivative **12a** was as active as the parent compound **1**. Further, the carbonyl moiety of the thienopyridazinone derivative was shown not to be necessary for the activities.



a) 2 eq of LDA, $-78\,°C$, 3-pyridinecarbaldehyde; b) $KMnO_4$; c) MeOH, HCl gas; d) $(COCl)_2$, DMSO, Et_3N, $-70\,°C$; e) H_2NNH_2, EtOH, reflux; f) EtBr, NaH; g) $POCl_3$ reflux; h) EtONa, EtOH.

Chart 2

Table I. TXA_2 Synthetase-Inhibitory and Bronchodilatory Activities

Compound	% inhibition of TXA_2 production		Bronchodilatory activity	
	In vitro at 1 μM	*Ex vivo*[a] 3 mg/kg *p.o.*	*In vitro*[b] $-\log[IC_{50}$ (M)$]$	*In vivo*[c] % inhibition
1[d]	87	66	5.88	72
7	80	53	5.20	ND
12a	85	84	5.79	89
12b	ND	37	4.72	65
19	ND	35	4.42	ND
21	83	80	5.35	ND
OKY-046[e]	89	86	<3.0	0
Aminophylline	0	0	4.33	23

a) At 1 h after oral administration of test compounds. *b*) Concentration activity curves were obtained with seven concentrations of test compounds, and IC_{50} values were calculated from the log curve. *c*) Inhibitory effects of test compounds on airway constriction induced by histamine (2—5 μg/kg, i.v.) at 30 min after 10 mg/kg intraduodenum administration of test compound. *d*) See reference 2. *e*) See reference 9.

Experimental

The melting points were measured with a Yanagimoto hot plate micro melting point apparatus and are uncorrected. The IR spectra were obtained with a Hitachi Model 270-30 infrared spectrometer. The ^1H-NMR spectra were taken with a Hitachi Model R-24B high-resolution magnetic resonance spectrometer (60 MHz) using tetramethylsilane as an internal standard. Mass spectra (MS) were obtained on a Shimadzu Model GCMS-QP1000 mass spectrometer and are reported as mass/charge ratio (relative intensity). Organic extracts were dried over anhydrous sodium sulfate and concentrated in a rotary evaporator.

6-Ethyl-4-(3-pyridyl)-thieno[2,3-*d*]pyridazin-7(6*H*)-one (7) A solution of 3-bromothiophene (**4**) (12.2 g, 75 mmol) in tetrahydrofuran (THF) (50 ml) was added dropwise to a solution of 1.5 M *n*-BuLi (in 50 ml hexane, 75 mmol) at $-78\,°C$ under a nitrogen atmosphere, and the mixture was stirred for 15 min at the same temperature. Then a solution of 3-cyanopyridine (8.0 g, 77 mmol) in THF (50 ml) was added rapidly at $-78\,°C$. The reaction mixture was stirred for an additional 1 h at $-78\,°C$, then allowed to warm to room temperature and further stirred for 1 h. It was cooled to $-5\,°C$, and a second aliquot of 1.5 M *n*-BuLi (in 50 ml hexane, 75 mmol) was added in one portion. The reaction mixture was heated at 40 °C for 2 h, cooled to $-70\,°C$, and slowly poured under a nitrogen atmosphere into a flask half filled with crushed dry ice (500 g). The whole was warmed to room temperature overnight and then poured into 3 N HCl solution. This mixture was stirred for 30 min at 40 °C, and washed with AcOEt. The aqueous layer was adjusted to pH 8 with $NaHCO_3$ and washed with AcOEt. The aqueous layer was adjusted to pH 4 with 3 N HCl solution and extracted with AcOEt. The extract was dried and concentrated to give 3.0 g of crude 3-(3-pyridinoyl)thiophene-2-carboxylic acid (**5**) as a yellow solid. A mixture of crude **5** (3.0 g, 13 mmol) and 80% hydrazine hydrate (1 g, 16 mmol) in EtOH (200 ml) was refluxed for 6 h. The mixture was cooled, concentrated and adjusted to pH 8 with diluted HCl, and the resulting precipitates were collected by filtration, washed with EtOH, and recrystallized from EtOH to give 1.2 g (7% from **4**) of 4-(3-pyridyl)thieno[2,3-*d*]pyridazin-7(6*H*)-one (**6**) as white crystals, mp 255—256 °C. IR (KBr): 1675 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 7.50—7.82 (2H, m), 8.09—8.54 (2H, m), 8.72—8.91 (1H, m), 8.97 (1H, d, J=4 Hz), 13.20 (1H, br s). MS m/z: 259 (M$^+$, 78), 228 (100). *Anal.* Calcd for $C_{11}H_7N_3OS \cdot 5/6H_2O$: C, 54.09; H, 3.55; N, 17.29. Found: C, 54.05; H, 3.36; N, 17.30. To a suspension of 60% NaH in oil (0.32 g, 7.9 mmol) in *N*,*N*-dimethylformamide (DMF) (100 ml) was added **6** (1.4 g, 6.1 mmol), and the mixture was stirred for 30 min at room temperature under a nitrogen atmosphere. The mixture was cooled to 0 °C, ethyl bromide (0.6 ml, 8 mmol) was added, and the whole was stirred for 1 h at room temperature. The mixture was poured into ice-water (150 ml) and extracted with AcOEt. The extract was dried, concentrated, and chromatographed on silica gel with $CHCl_3$–MeOH (99 : 1) to give 0.9 g (57%) of **7** as white crystals, mp 125—127 °C ($CHCl_3$–hexane). IR (KBr): 1645 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.50 (3H, t, J=7 Hz), 4.41 (2H, q, J=7 Hz), 7.40—7.74 (2H, m), 7.78—8.43 (2H, m), 8.72—9.31 (2H, m). MS m/z: 257 (M$^+$, 100). *Anal.* Calcd for $C_{13}H_{11}N_3OS$: C, 60.68; H, 4.31; N, 16.33. Found: C, 60.85; H, 4.12; N, 15.29.

In a similar manner, 6-ethyl-4-(3-pyridyl)-thieno[2,3-*d*]pyridazin-7(6*H*)-one (**12a**) was prepared from 1,2-dibromothiophene (**8**) *via* 4-(3-pyridyl)-thieno[2,3-*d*]pyridazin-7(6*H*)-one (**11a**). We were unable to purify **10a**. **11a** was obtained as white crystals, yield 10% from **8**, mp 286—288 °C (EtOH). IR (KBr): 1675 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 7.51—7.94 (2H, m), 8.10—8.48 (2H, m), 8.71—8.92 (1H, m), 9.10 (1H, d, J=4 Hz), 13.20 (1H, br s). MS m/z: 259 (M$^+$, 82), 228 (100). *Anal.* Calcd for $C_{11}H_7N_3OS$: C, 57.63; H, 3.08; N, 18.33. Found: C, 57.31; H, 2.83; N, 18.26. **12a** was obtained as white crystals, yield 69%. mp 107—108 °C ($CHCl_3$–hexane). IR (KBr): 1645 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.52 (3H, t, J=7 Hz), 4.40 (2H, q, J=7 Hz), 7.31—8.04 (3H, m), 8.10—8.42 (1H, m), 8.70—8.91 (1H, m), 9.22—9.43 (1H, m). MS m/z: 257 (M$^+$, 67), 40 (100). *Anal.* Calcd for $C_{13}H_{11}N_3OS$: C, 60.68; H, 4.31; N, 16.33. Found: C, 60.83; H, 4.20; N, 16.31.

2-(3-Pyridinoyl)thiophene-3-carboxylic Acid (10a) A 1.5 M lithium diisopropylamide (in 57 ml cyclohexane, 86 mmol) was rapidly added dropwise to a solution of 3-thiophenecarboxylic acid (**9a**) (5.0 g, 39 mmol) in THF (200 ml) at 0 °C under a nitrogen atmosphere. 3-Pyridinecarbaldehyde (4.1 ml, 43 mmol) was added to the mixture at 0 °C, and the whole was stirred for 3 h at room temperature. The reaction was quenched by the addition of ice-water (100 ml), and the mixture was concentrated under reduced pressure and washed with AcOEt. To the residual aqueous layer, $KMnO_4$ powder (12.4 g, 78 mmol) was added in portions, and the mixture was stirred for 3 h at 60 °C. It was then filtrated and washed with hot water, and the resulting filtrate was adjusted to pH 4 with diluted HCl. The resulting precipitates were collected by filtration, washed with water, and dried to give 6.8 g (75%) of **10a** as a white solid. ^{1}H-NMR ($CDCl_3$) δ: 7.32—7.65 (2H, m), 7.74—8.22 (2H, m), 8.63—8.88 (2H, m). MS m/z: 233 (M$^+$, 58), 216 (100). **11a** was prepared from **10a** in the same way using hydrazine hydrate as described above. The analytical data of **11a** obtained by this method agreed with those of **11a** prepared from **8**.

In a similar manner, 4-methoxy-2-(3-pyridinoyl)thiophene-3-carboxylic acid (**10b**) and 3-methoxy-5-ethyl-7-(3-pyridyl)-thieno[2,3-*d*]pyridazin-4(5*H*)-one (**12b**) were prepared from 4-methoxy-3-thiophenecarboxylic acid (**9b**). **10b** was obtained as a white solid, yield 53% from **9b**. ^{1}H-NMR (DMSO-d_6) δ: 3.83 (3H, s), 7.09 (1H, s), 7.35—7.60 (1H, m), 7.92—8.30 (1H, m), 8.60—8.96 (1H, m). MS m/z: 263 (M$^+$, 46), 78 (100). **11b** was obtained as white crystals, yield 75%, mp 250 °C (dec.) (EtOH). IR (KBr): 1675 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 3.95 (3H, s), 7.02 (1H, s), 7.40—7.76 (1H, m), 8.03—8.28 (1H, m), 8.64—8.85 (1H, m), 8.90—9.13 (1H, m), 13.50 (1H, br s). **12b** was obtained as white crystals, yield 87%, mp 186—188 °C ($CHCl_3$–hexane). IR (KBr): 1645 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.50 (3H, t, J=7 Hz), 4.03 (3H, s), 4.45 (2H, q, J=7 Hz), 6.54 (1H, s), 7.18—7.49 (1H, m), 8.00—8.21 (1H, m), 8.58—8.71 (1H, m), 9.02—9.13 (1H, m). MS m/z: 287 (M$^+$, 100). *Anal.* Calcd for $C_{14}H_{13}N_3O_2S$: C, 58.52; H, 4.56; N, 14.62. Found: C, 58.24; H, 4.55; N, 14.61.

5-Ethyl-7-(3-pyridyl)-furo[2,3-*d*]pyridazin-4(5*H*)-one (19) A 1.5 M lithium diisopropylamide solution (in 67 ml of cyclohexane, 100 mmol) was added dropwise to a solution of 3-furancarboxylic acid (**13**) (5.0 g. 45 mmol) in THF (200 ml) at −78 °C under a nitrogen atmosphere. A solution of 3-pyridinecarbaldehyde (4.7 ml, 50 mmol) in THF (10 ml) was added at −78 °C, and the whole was stirred for 1 h at the same temperature. It was allowed to warm to room temperature and the reaction was quenched by the addition of ice-water (200 ml). The mixture was concentrated under reduced pressure, and the resulting aqueous layer was washed with AcOEt and adjusted to pH 4 with diluted HCl. The aqueous layer was saturated with NaCl and extracted with THF. The extract was dried and concentrated under reduced pressure to give 9.5 g (97%) of 2-[hydroxy-(3-pyridyl)methyl]furan-3-carboxylic acid (**14**) as a white solid. ^{1}H-NMR (DMSO-d_6) δ: 6.44 (1H, s), 6.56—6.64 (1H, m), 7.15—7.42 (1H, m), 7.48—7.59 (1H, m), 7.62—7.86 (1H, m), 8.30—8.57 (1H, m). MS m/z: 219 (M$^+$, 53), 145 (100). A suspension of **14** (9.5 g, 43 mmol) in MeOH (150 ml) was saturated with HCl gas and stirred overnight at room temperature. The mixture was concentrated and the residue was taken up in a mixture of saturated aqueous $NaHCO_3$ and $CHCl_3$. The organic layer was separated, dried, and concentrated. The residual oil was chromatographed on silica gel with $CHCl_3$–MeOH (20 : 1) to give 8.9 g (88%) of methyl 2-[hydroxy-(3-pyridyl)methyl]furan-3-carboxylate (**16**) as an oil. IR (KBr): 3150, 1720 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 3.80 (3H, s), 6.05 (1H, br s), 6.32 (1H, s), 6.60—6.72 (1H, m), 7.05—7.37 (2H, m), 7.68—7.92 (1H, m), 8.34—8.60 (2H, m). A solution of dimethyl sulfoxide (5.36 ml, 76 mmol) in CH_2Cl_2 (18 ml) was added dropwise to a solution of oxalyl chloride (3.4 ml, 3.9 mmol) in CH_2Cl_2 (90 ml) at −70 °C under a nitrogen atmosphere, and the mixture was stirred for 2 min at the same temperature. A solution of **16** (8.0 g, 34 mmol) in CH_2Cl_2 (35 ml) was added to the mixture at the same temperature and the whole was stirred for 15 min. Triethylamine (24 ml, 172 mmol) was added and the mixture was stirred for another 15 min. The mixture was allowed to warm to room temperature and the reaction was quenched by the addition of water (150 ml). The organic layer was separated, dried and concentrated under reduced pressure. The residual oil was chromatographed on silica gel with $CHCl_3$–MeOH (20 : 1) to give 7.3 g (92%) of methyl 2-(3-pyridinoyl)thiophene-3-carboxylate (**17**) as an oil. ^{1}H-NMR ($CDCl_3$) δ: 3.66 (3H, s), 6.77—6.90 (1H, m), 7.25—7.64 (2H, m), 8.00—8.26 (1H, m), 8.75—9.17 (2H, m). Cyclization and ethylation of **17** gave 7-(3-pyridyl)-furo[2,3-*d*]pyridazin-4(5*H*)-one (**18**) and 5-ethyl-7-(3-pyridyl)-furo[2,3-*d*]pyridazin-4(5*H*)-one (**19**). **18** as white crystals, yield 85%, mp 254—255 °C (EtOH). ^{1}H-NMR (CD_3OD) δ: 7.12 (1H, d, J=2 Hz), 7.38—7.60 (1H, m), 7.80 (1H, d, J=2 Hz), 8.29—8.58 (2H, m), 9.20—9.32 (1H, m). MS m/z: 213 (M$^+$, 100). *Anal.* Calcd for $C_{11}H_9N_3O_2$: C, 61.97; H, 3.31; N, 19.71. Found: C, 61.60; H, 3.20; N, 19.73. **19** was obtained as white crystals, yield 66%, mp 109—110 °C ($CHCl_3$–hexane). ^{1}H-NMR ($CDCl_3$) δ: 1.51 (3H, t, J=7 Hz), 4.40 (2H, q, J=7 Hz), 7.10 (1H, d, J=2 Hz), 7.19—7.62 (1H, m), 7.72 (1H, d, J=2 Hz), 8.20—8.51 (1H, m), 8.62—8.84 (1H, m), 9.22—9.33 (1H, m). MS m/z: 241 (M$^+$, 100). *Anal.* Calcd for $C_{13}H_{11}N_3O_2$: C, 64.72; H, 4.60; N, 17.42. Found: C, 64.72; H, 4.51; N, 17.53.

4-Ethoxy-7-(3-pyridyl)-thieno[2,3-*d*]pyridazine (21) A suspension of **11a** (6 g, 26 mmol) in $POCl_3$ (60 ml) was refluxed for 3 h. The mixture was concentrated and the residue was washed with hexane, dissolved in a small amount of water and adjusted to pH 8 with 1 N NaOH and saturated $NaHCl_3$ solution. The resulting precipitates were collected and dried to give 5.2 g (81%) of 4-chloro-7-(3-pyridyl)thieno[2,3-*d*]pyridazine (**20**) as a white solid, mp 210—211 °C. IR (KBr): 1360 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 7.35—7.60 (1H, m), 7.68 (1H, d, J=7 Hz), 7.98 (1H, d, J=7 Hz), 8.25—8.53 (1H, m), 8.67—8.87 (1H, m), 9.26—9.40 (1H, m). MS m/z: 247 (M$^+$, 72), 212 (100). A solution of **20** (2.1 g, 8.3 mmol) in EtOH (50 ml) was added to a solution of sodium (0.7 g, 30 mmol) in EtOH (50 ml). The mixture was stirred for 12 h at room temperature, then for 3 h at 50 °C, water (50 ml) was added, and the whole was extracted with $CHCl_3$. The extact was dried and concentrated. The residual solid was recrystallized from $CHCl_3$–hexane to give 1.5 g (69%) of **21** as white crystals, mp 131—133 °C. IR (KBr): 1405 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.60 (3H, t, J=7 Hz), 7.82 (2H, q, J=7 Hz), 7.23—8.04 (3H, m), 8.32—8.58 (1H, m), 8.62—8.91 (1H, m), 9.27—9.51 (1H, m). MS m/z: 257 (M$^+$, 73), 242 (100). *Anal.* Calcd for $C_{13}H_{11}N_3OS$: C, 60.68; H, 4.31; N, 16.33. Found: C, 60.73; H, 4.28; N, 16.24.

In Vitro* Enzyme Assay of TXA_2 Synthetase** Rabbit platelet microsomes as the enzyme source were prepared according to the method of Needleman *et al.*[8] A reaction mixture (15 mM Tris–HCl, 140 mM NaCl, 10 mM glucose, pH 7.6) containing rabbit platelets (*ca.* 10^8/ml) was preincubated with each test compound (10^{-6} M) for 3 min at 25 °C. After addition of arachidonic acid (1—3 μM), the reaction mixture was incubated for a further 3 min at 25 °C. The reaction was terminated by chilling and adding an appropriate amount of 1 N HCl to bring the pH of the reaction mixture to 3. After centrifugation at 1500 × ***g for 10 min at 4 °C, the content of TXB_2 in the supernatant was measured with a

TXB_2 radioimmunoassay kit (Amersham). As a control, a reaction mixture was preincubated with the vehicle and the subsequent reactions were carried out as described. The percent inhibition of TXA_2 synthetase was calculated as relative to the content of TXB_2 in the control (5.5 ng/tube).

***Ex Vivo* Effect on Serum TXB_2 Concentration** Male SD rats (240—260 g) were starved for 20 h and dosed orally with test compounds (dissolved or suspended in 0.5% carboxymethylcellulose) or the vehicle. At 1 h after administration, the rats were anesthetized with ether, and blood (2 ml) was withdrawn from the heart and allowed to clot at 37 °C for 90 min. The clotting blood was centrifuged to obtain the serum. The serum was deproteinized with EtOH and the resulting supernatant was stored at −20 °C. The serum TXB_2 concentration was measured with a TXB_2 radioimmunoassay kit (Amersham). The percent inhibition was calculated as the decrease in the serum TXB_2 concentration compared to each control group (380 ng/ml).

Relaxing Effect on Guinea Pig Isolated Tracheal Strips Guinea pig tracheal strips were suspended under isotonic conditions (TD-112S, Nihon Kohden, Tokyo) in oxygenated Krebs-Henseleit solution. Tension was allowed to develop spontaneously and was set at 1 g in the presence of aminophylline (10^{-3} M). Compounds were added in a cumulative fashion up to a maximum concentration of 100 μM and the relaxant effects were calculated as a percentage of the relaxation induced by aminophylline (10^{-3} M) added at the end of the experiment. The IC_{50} value of each compound was defined as the concentration which produced 50% of the response to aminophylline as measured from the concentration–response curve, and generally (apart from compounds which had IC_{50} values of $>100\ \mu$M) was the mean of three or more determinations. Each IC_{50} value is expressed as a negative logarithm.

Effect on Bronchoconstriction Induced by Histamine in Guinea Pigs Male Dunkin-Hartley guinea-pigs were anesthetized with i.p.-injected pentobarbital (35 mg/kg). The jugular vein and trachea were cannulated and the animals were artificially ventilated (10 mg/kg, 60 strokes/min). The pressure in the respirator system, *i.e.* the insufflation pressure, was measured constantly with a pressure transducer (P10EZ-1, Nihon Kohden, Tokyo). Histamine (2—5 μg/kg) was injected i.v. every 10 min through the jugular vein cannula to induce bronchoconstriction and administered repeatedly until a reproducible constriction (control response) was obtained. Test compound (10 mg/kg) was administered intraduodenally 1 min before another challenge with histamine. The inhibitory effect of each compound was determined from three or more experiments as the percent inhibition compared to the control response and expressed as a mean.

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