Novel Antiasthmatic Agents with Dual Activities of Thromboxane A_2 Synthetase Inhibition and Bronchodilation. III.[1] 4-[2-(5-Ethyl-2-thienyl)]-2′-[2-(1-imidazolyl)ethyl]-1(2*H*)-phthalazinones

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Synthesis and pharmacological evaluation of novel 4-[2-(5-ethyl-2-thienyl)]-2-[2-(1-imidazolyl)ethyl]-1(2*H*)-phthalazinones are described. The phenyl moiety of the phthalazinone skeleton was found to play an important role in both thromboxane A_2 synthetase-inhibitory and bronchodilatory activities.

Keywords phthalazinone; TXA_2 synthetase inhibitor; bronchodilator; antiasthmatic agent

We have synthesized a number of 2,4-disubstituted 1(2*H*)-phthalazinones in order to develop novel agents possessing both thromboxane A_2 (TXA_2) synthetase-inhibitory and bronchodilatory activities.[1,2] In a previous paper, we reported the synthesis of 4-substituted 2-(ω-1-imidazolyl)alkyl-1(2*H*)-phthalazinones and examined the relationship between activities and 2- or 4-substituted structure. We found that 4-[2-(5-ethyl-2-thienyl)]-2-[2-(1-imidazolyl)ethyl]-1(2*H*)-phthalazinone (**1**) is a potent agent with well-balanced dual activities.[2] However, the role of the phenyl moiety of the phthalazinone skeleton remains to be elucidated. In order to explore this aspect, we prepared 5,6,7,8-tetrahydro- (**5**) and some 6- or 7-substituted derivatives (**9a**—**e**) of **1** and evaluated their pharmacological activities in comparison with those of **1**.

Preparation of these compounds was performed by use of the following reaction sequence, involving the Friedel–Crafts acylation of 2-ethylthiophene with phthalic anhydride derivatives, cyclization with hydrazine hydrate and 2-(1-imidazolyl)ethylation with 1-(2-bromoethyl)imidazole. Acylation of 2-ethylthiophene with 3,4,5,6-tetrahydrophthalic anhydride (**2**) smoothly occurred, and **5** was obtained in practically the same manner as **1** (Chart 1).

Acylation of 2-ethylthiophene with 4-hydroxyphthalic anhydride (**6a**) led to a mixture of the 4- and 5-hydroxy-2-thenoylbenzoic acids (**7a**), which was used for the next reaction without separation. Methylation of **7a** with iodomethane provided a mixture of the 4- and 5-methoxy derivatives (**7b**), which was converted *via* a mixture of the methoxyphthalazinones (**8a**) to a mixture of the 6- (**9a**) and 7-methoxy (**9b**) derivatives of **1**; separation of this mixture into **9a** and **9b** was easily effected by silica gel column chromatography with $CHCl_3$–MeOH (20 : 1). The position of each methoxy group was determined from the chemical shift and coupling constant of the proton at the 8-position in ^{1}H-NMR analysis. On the other hand, acylation with 4-nitro- and 4-carboxylic-phthalic anhy-

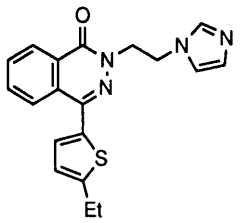

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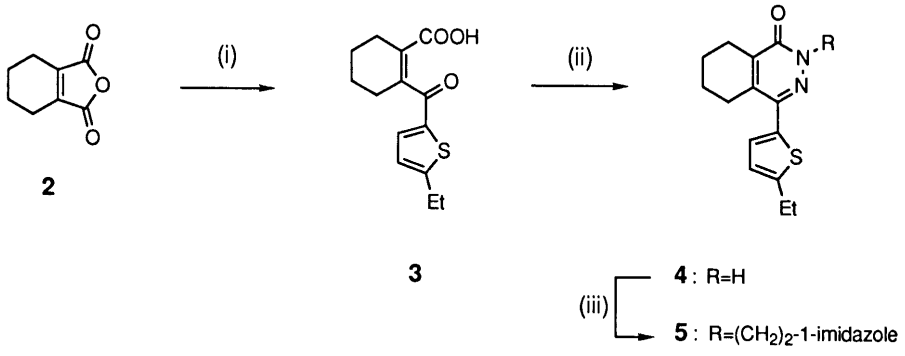
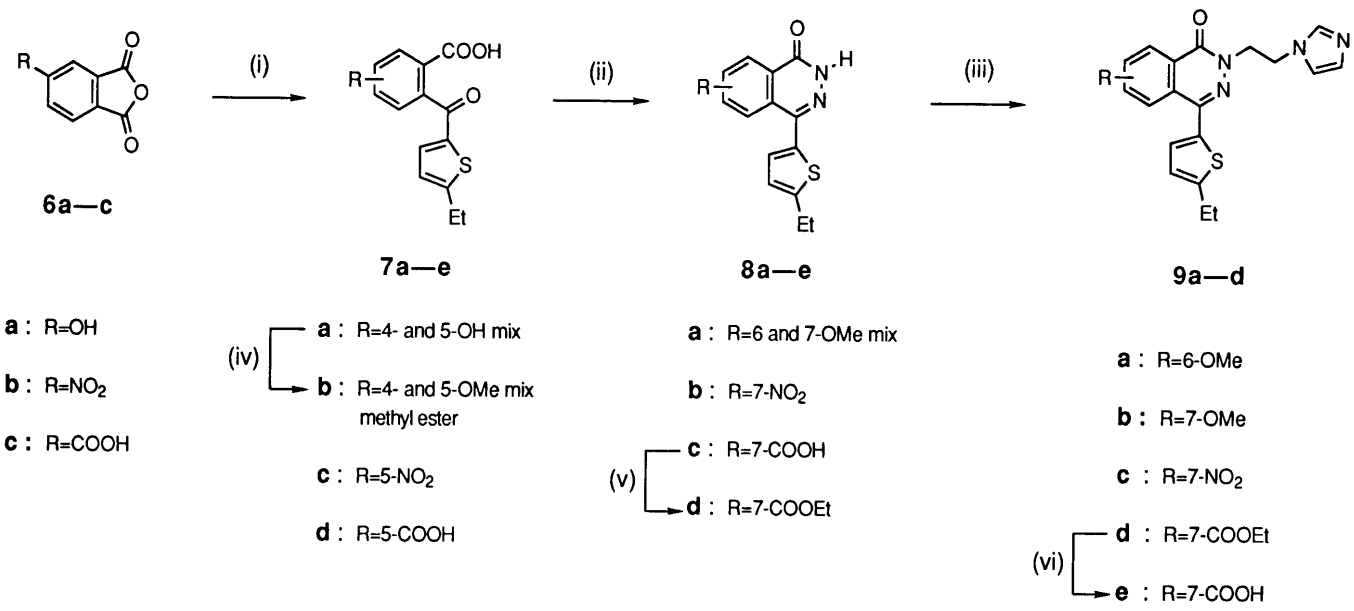
Fig. 1. 2-[2-(1-Imidazolyl)ethyl]-4-[2-(5-ethyl-2-thienyl)]-1(2*H*)-phthalazinone



2 **3**

(i) 2-ethylthiophene, $AlCl_3$; (ii) H_2NNH_2, EtOH, reflux; (iii) 1-(2-bromoethyl)imidazole, K_2CO_3, 80 °C

Chart 1



(i) 2-ethylthiophene, $AlCl_3$; (ii) H_2NNH_2, EtOH, reflux; (iii) 1-(2-bromoethyl)imidazole, K_2CO_3, 80 °C; (iv) iodomethane, K_2CO_3; EtOH, H_2SO_4, reflux; (v) NaOH aq.

Chart 2

dride (**6b**, **c**) afforded the corresponding 5-substituted benzoic acids (**7c**, **d**) without any detectable 4-substituted isomer. From **7c**, the 7-nitro derivative of **1** (**8b**) was prepared in the same way. The 7-carboxy-4-thienylphthalazinone (**8c**) obtained from **7d** was esterified with ethanol and sulfuric acid, followed by transformation into the 7-ethoxycarbonyl derivative of **1** (**9d**). Alkaline hydrolysis of **9d** yielded the 7-carboxy compound (**9e**) (Chart 2).

In order to test for TXA_2 synthetase-inhibitory activity, TXA_2 synthetase inhibition with rabbit enzyme was employed as an *in vitro* assay, and rat serum TXA_2 production as an *ex vivo* assay. None of these compounds inhibited PGI_2 formation. These results are consistent with a mechanism of selective TXA_2 synthetase inhibition. To test for bronchodilatory activity, we employed spontaneous tone inhibition with guinea pig tracheal strips as an *in vitro* assay and inhibitory effect on histamine-induced bronchoconstriction using anesthetized guinea pigs as an *in vivo* assay. Further, we used OKY-046[3] for TXA_2 synthetase inhibition and aminophylline for bronchodilation as active controls.

The 5,6,7,8-tetrahydrophthalazinone derivative **5** exhibited no significant TXA_2 synthetase-inhibitory activity, although its *in vitro* bronchodilatory activity was comparable with that of the parent compound **1** (Table I). Introduction of a methoxy group (**9a**, **b**) resulted in the reduction of both *in vitro* activities. While the 7-nitro derivative **9c** showed no significant TXA_2 synthetase-inhibitory activity, it demonstrated the most effective bronchodilatory activity among the test compounds in this study. Introduction of not only a carboxy group (**9e**) but also an ester group (**9d**) afforded a higher *in vivo* TXA_2 synthetase-inhibitory activity than that of the parent compound **1**. Since a carboxylic acid group, in general, is considered to be effective for TXA_2 synthetase-inhibitory activity,[4] it is not surprising that **9e**, a carboxylic acid derivative, exhibited a relatively high TXA_2 synthetase-inhibitory activity *in vitro*. On the other hand, it is rather noteworthy that an ester derivative (**9d**) retained the TXA_2 synthetase-inhibitory activity, though the reason for this is not yet clear. These two compounds exhibited no significant activity in *ex vivo* assay, contrary to our expectations. As for bronchodilatory activity, neither **9e** nor **9d** exhibited any significant potency in either the *in vitro* or *in vivo* test. This observation is consistent with the previous finding that introduction of a carboxy group led to a reduction in bronchodilatory activity.[2] All of these results indicated that the phenyl moiety of the phthalazinone skeleton plays an important role in both activities, in particular TXA_2 synthetase-inhibitory activity. Further, introduction of a substituent into the 6- or 7-position of the phthalazinone skeleton decreased

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] 30 mg/kg *p.o.*	*In vitro*[b] $-\log[IC_{50}(M)]$	*In vivo*[c] % inhibition
1[d]	57	52	5.75	96
5	15		5.69	45
9a	35		5.36	100
9b	40	76	5.14	89
9c	27	9	6.19	
9d	87	27	4.29	48
9e	87	27	4.76	13
OKY-046[e]	89	92	<3.0	0
Aminophylline	0	0	4.33	86

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 1 min after 10 mg/kg i.v. administration of test compounds. *d*) See reference 2. *e*) See reference 3.

the TXA_2 synthetase-inhibitory activity, except for the carboxylic group. These significant decreases in TXA_2 synthetase-inhibitory activity may conceivably be due to the steric effects of the substituents on the binding to TXA_2 synthetase.

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 ^{1}H-NMR spectra were taken with a Hitachi Model R-24B high-resolution magnetic resonance spectrometer (60 MHz) using tetramethylsilane or sodium 2,2-dimethyl-2-silapentane-5-sulfonate (in D_2O) as an internal standard. Organic extracts were dried over anhydrous sodium sulfate and concentrated in a rotary evaporator.

4-[2-(5-Ethyl-2-thienyl)]-2-[2-(1-imidazolyl)ethyl]-5,6,7,8-tetra-hydro-1(2*H*)-phthalazinone (5) $AlCl_3$ (26 g, 200 mmol) was added in portions to a solution of 3,4,5,6-tetrahydrophthalic anhydride (**2**) (25 g, 166 mmol) in CH_2Cl_2 (150 ml) was added at 0 °C. A solution of 2-ethylthiophene (23 g, 200 mmol) in CH_2Cl_2 (40 ml) was added, and the mixture was stirred for 3 h at room temperature. The reaction mixture was poured into diluted HCl solution and extracted with 500 ml of $CHCl_3$. The extract was shaken with 5% K_2CO_3, and the alkaline washings were made acidic with diluted HCl solution then extracted with $CHCl_3$. The extract was dried and concentrated under reduced pressure. The residual oil was chromatographed on silica gel with $CHCl_3$–MeOH (20 : 1) to give 22 g (51%) of 2-[2-(5-ethylthenoyl)]-3,4,5,6-tetrahydrobenzoic acid (**3**) as a pale yellow oil. IR (neat): 1640, 1690 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.28 (3H, t, J=7 Hz), 2.10—3.00 (8H, m), 2.86 (2H, q, J=7 Hz), 6.85 (1H, d, J=4 Hz), 7.49 (1H, d, J=4 Hz), 11.82 (1H, br s). A solution of **3** (2 g, 7.6 mmol) and 80% hydrazine hydrate (0.7 g, 11.4 mmol) in EtOH (100 ml) was refluxed for 6 h. After cooling, the mixture was concentrated. To the residual oil, AcOEt and water were added, and the organic layer was separated. The extract was washed with brine, dried, and concentrated under reduced pressure. The residue was chromatographed on silica gel with $CHCl_3$ to give 0.8 g (40%) of 4-[2-(5-ethyl-2-thienyl)]-5,6,7,8-tetrahydro-1(2*H*)-phthalazinone (**4**) as a yellow solid, mp 220—222 °C. IR (KBr): 1660 cm^{-1}. ^{1}H-NMR ($CDCl_3$–DMSO-d_6 1 : 1) δ: 1.28 (3H, t, J=7 Hz), 1.55—2.02 (8H, m), 2.83 (2H, q, J=7 Hz), 6.70 (1H, d, J=4 Hz), 7.08 (1H, d, J=4 Hz), 11.2 (1H, br s). A mixture of **4** (1.4 g, 5 mmol), 1-(2-bromoethyl)imidazole hydrogen bromide (1.5 g, 6 mmol), and K_2CO_3 (2.7 g, 20 mmol) in *N*,*N*-dimethylformamide (DMF) (50 ml) was stirred for 3 h at 70 °C. The mixture was cooled, and 2 N HCl and AcOEt were added thereto. The acidic aqueous layer was separated, made alkaline with 5% K_2CO_3 solution, and extracted with AcOEt (200 ml). The extract was washed with brine, dried, and concentrated under reduced pressure. The residual oil was chromatographed on silica gel with $CHCl_3$–MeOH (20 : 1) to give 0.7 g (39%) of **5** as a pale yellow oil. IR (neat): 1675 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.30 (3H, t, J=7 Hz), 1.50—2.15 (8H, m), 2.81 (2H, q, J=7 Hz), 4.07—4.39 (4H, m), 6.64 (1H, d, J=4 Hz), 6.76—6.97 (2H, m), 7.02 (1H, d, J=4 Hz), 7.28 (1H, s). Treatment of **5** with HCl gas in EtOH afforded the HCl salt of **5** as a white solid, mp 161—163 °C. *Anal.* Calcd for $C_{19}H_{22}N_4OS \cdot HCl$: C, 58.38; H, 5.93; N, 14.33. Found: C, 58.21; H, 5.84; N, 14.30.

4-[2-(5-Ethyl-2-thienyl)]-2-[2-(1-imidazolyl)ethyl]-6-methoxy-1(2*H*)-phthalazinone (9a) and 4-[2-(5-Ethyl-2-thienyl)]-2-[2-(1-imidazolyl)ethyl]-7-methoxy-1(2*H*)-phthalazinone (9b) Similar treatment of 4-hydroxyphthalic anhydride (**6a**) with 2-ethylthiophene afforded a mixture of 4-hydroxy- and 5-hydroxy-2-[2-(5-ethylthenoyl)]benzoic acid (**7a**), and this was used for next reaction without separation. A suspension of **7a** (25 g, 91 mmol), iodomethane (39 g, 28 mmol), and K_2CO_3 (38 g, 28 mmol) in DMF (150 ml) was stirred overnight at room temperature. The mixture was poured into water and extracted with AcOEt. The extract was washed, dried, concentrated under reduced pressure. A mixture of the residual oil (**7b**, 24 g, 80 mmol) and 80% hydrazine hydrate (10 g, 16 mmol) in EtOH (150 ml) was refluxed for 4 h. After cooling, the resulting precipitates were collected, washed with EtOH and dried to give 12.6 g of a mixture of 6-methoxy- and 7-methoxy-4-[2-(5-ethyl-2-thienyl)]-1(2*H*)-phthalazinones (**8a**) as a pale yellow solid, mp 174—192 °C. IR (KBr): 1650 cm^{-1}. Treatment of **8a** with 1-(2-bromoethyl)imidazole afforded a mixture of **9a** and **9b** as a solid. The mixture was chromatographed on silica gel with $CHCl_3$–MeOH (20 : 1) to give successively **9a** (48%) and **9b** (29%). **9a**: white crystals, mp 139—141 °C ($CHCl_3$–hexane). IR (KBr): 1640 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.36 (3H, t, J=7 Hz), 2.88 (2H, q, J=7 Hz), 3.85 (3H, s), 4.27—4.60 (4H, m), 6.71—7.52 (7H, m), 8.29 (1H, d, J=9 Hz). *Anal.* Calcd for $C_{20}H_{20}N_4O_2S$: C, 63.13; H, 5.30; N, 14.73. Found: C, 63.11; H, 5.34; N, 14.70. **9b**: white crystals, mp 99—101 °C ($CHCl_3$–hexane). IR: 1650 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.35 (3H, t, J=7 Hz), 2.86 (2H, q, J=7 Hz), 3.91 (3H, s), 4.30—4.72 (4H, m), 6.70—8.14 (8H, m). *Anal.* Calcd for $C_{20}H_{20}N_4O_2S$: C, 63.13; H, 5.30; N, 14.73. Found: C, 62.95; H, 5.41; N, 14.69.

4-[2-(5-Ethyl-2-thienyl)]-2-[2-(1-imidazolyl)ethyl]-7-nitro-1(2*H*)-phthalazinone (9c) Treatment of 4-nitrophthalic anhydride (**6b**) with 2-ethylthiophene afforded crude 2-[2-(5-ethylthenoyl)]-5-nitrobenzoic acid (**7c**) (55%) as a brown oil. The resulting oil was treated with 80% hydrazine hydrate to give the corresponding 4-[2-(5-ethyl-2-thienyl)]-7-nitro-1(2*H*)-phthalazinone (**8b**) (48%) as a yellow solid, mp 184—187 °C. IR (KBr): 1655, 1520, 1340 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.33 (3H, *t*, J=7 Hz), 2.93 (2H, q, J=7 Hz), 6.98 (1H, d, J=4 Hz), 7.41 (1H, d, J=4 Hz), 7.92—8.43 (2H, m), 8.98 (1H, d, J=2 Hz), 12.90 (1H, br s). Introduction of an imidazolylethyl group gave rise to **9c** (74%) as a yellow solid, mp 124—125 °C ($CHCl_3$–hexane). IR (KBr): 1660, 1530, 1340 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ 1.37 (3H, t, J=7 Hz), 2.90 (2H, q, J=7 Hz), 4.38—4.62 (4H, m), 6.71—7.44 (5H, m), 8.02—8.64 (2H, m), 9.21 (1H, d, J=2 Hz). *Anal.* Calcd for $C_{19}H_{17}N_5O_3S$: C, 57.71; H, 4.33; N, 17.71. Found: C, 57.78; H, 4.24; N, 17.62.

7-Carboxy-4-[2-(5-ethyl-2-thienyl)]-2-[2-(1-imidazolyl)ethyl]-1(2*H*)-phthalazinone (9e) Treatment of 4-carboxyphthalic anhydride (**6c**) with 2-ethylthiophene afforded 5-carboxy-2-[2-(5-ethylthenoyl)]benzoic acid (**7d**) (87%) as a brown solid, mp 183—185 °C. IR (KBr): 3700—2300, 1705, 1650 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.31 (3H, t, J=7 Hz), 2.92 (2H, q, J=7 Hz), 6.91 (1H, d, J=4 Hz), 7.10 (1H, d, J=4 Hz), 7.50—7.87 (2H, m), 8.12 (1H, d, J=2 Hz) 8.55 (2H, br s). Cyclization of **7d** using 80% hydrazine hydrate gave 7-carboxy-4-[2-(5-ethyl-2-thienyl)]-1(2*H*)-phthalazinone (**8c**) (61%) as a pale yellow solid, mp 272—275 °C. IR (KBr): 3700—2300, 1700, 1655 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.34 (3H, t, J=7 Hz), 2.94 (2H, q, J=7 Hz), 7.00 (1H, d, J=4 Hz), 7.43 (1H, d, J=4 Hz), 8.20—8.56 (2H, m), 8.93 (1H, d, J=2 Hz), 13.00 (1H, br s). Concentrated H_2SO_4 (0.3 ml) was added to a solution of **8c** (2 g, 6.6 mmol) in EtOH (150 ml), and the mixture was refluxed for 12 h. The solvent was evaporated off, 5% K_2CO_3 solution (150 ml) was added, and the resulting precipitates were collected, washed, dried and recrystallized from $CHCl_3$–hexane to give 1.6 g (88%) of 7-ethoxycarbonyl-4-[2-(5-ethyl-2-thienyl)]-1(2*H*)-phthalazinone (**8d**) as white crystals, mp 208—210 °C ($CHCl_3$–EtOH). IR (KBr): 1730, 1660 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.32 (3H, t, J=7 Hz), 1.45 (3H, t, J=7 Hz), 2.88 (2H, q, J=7 Hz), 4.41 (2H, q, J=7 Hz), 6.78 (1H, d, J=4 Hz), 7.22 (1H, d, J=4 Hz), 8.15 (1H, d, J=9 Hz), 8.38 (1H, dd, J=9, 2 Hz), 9.03 (1H, d, J=2 Hz) 12.88 (1H, br s). Introduction of an imidazolylethyl group by the method described above afforded 7-ethoxycarbonyl-4-[2-(5-ethyl-2-thienyl)]-2-[2-(1-imidazolyl)ethyl]-1(2*H*)-phthalazinone (**9d**) (58%) as white crystals, mp 133—135 °C ($CHCl_3$–hexane). IR (KBr): 1710, 1660 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.36 (3H, t, J=7 Hz), 1.41 (3H, t, J=7 Hz), 2.89 (2H, q, J=7 Hz), 4.20—4.73 (6H, m), 6.68—7.47 (5H, m), 7.93—8.54 (2H, m), 9.00 (1H, d, J=2 Hz). *Anal.* Calcd for $C_{22}H_{22}N_4O_3S \cdot 1/4H_2O$: C, 61.88; H, 5.31; N, 13.12. Found: C, 61.92; H, 5.19; N, 13.00. A mixture of **9d** (0.7 g, 2.2 mmol) and 1 N NaOH (4.4 ml, 4.4 mmol) in EtOH (50 ml) was stirred overnight at room temperature. The mixture was adjusted to pH 7 with diluted HCl, and the resulting precipitates were collected, washed with EtOH, and dried to give 0.6 g (69%) of **9e** as a white solid, mp >300 °C. IR (KBr): 3700—2300, 1660 cm^{-1}. ^{1}H-NMR (D_2O) δ: 1.02 (3H, t, J=7 Hz), 2.47 (2H, q, J=7 Hz), 4.17 (4H, br s), 6.20—8.24 (7H, m), 8.41 (1H, s). *Anal.* Calcd for $C_{20}H_{18}N_4O_3S \cdot 1/2H_2O$: C, 59.54; H, 4.75; N, 13.89. Found: C, 59.61; H, 4.70; N, 13.73.

***In Vitro* Enzyme Assay of TXA_2 Synthetase** Rabbit platelet microsomes as the enzyme source were prepared according to the methods of Needleman.[5] 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, the reaction mixture was preincubated with the vehicle and the subsequent reactions carried out as previously described. The percent inhibition of TXA_2 synthetase was calculated relative to the content of TXB_2 in the control.

***Ex Vivo* Effects 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 clotted 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 the respective control group.

Relaxing Effect on Guinea Pig Isolated Tracheal Strips Guinea pig tracheal strips were suspended under isotonic conditions in oxygenated Krebs–Henseleit solution. Tension was allowed to develop spontaneously and resting tension 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 relaxing 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 taken as the concentration which produced 50% of the response to aminophylline as measured from the concentration–response curve, and was generally (apart from compounds which had IC_{50} values of > 100 μM) the mean of three or more determinations. Each IC_{50} value is expressed as a negative logarithm.

Effects 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 ml/kg, 60 strokes/min). The pressure in the respirator system, *i.e.* the insufflation pressure, was measured continuously with a pressure transducer. Histamine (1—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. A test compound (10 mg/kg) was administered i.v. 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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