Novel Antiasthmatic Agents with Dual Activities of Thromboxane A_2 Synthetase Inhibition and Bronchodilation. IV.[1] 2-[2-(1-Imidazolyl)ethyl]-4-(3-pyridyl)-1(2*H*)-phthalazinones

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Synthesis and pharmacological evaluation of several compounds related to 2-[2-(1-imidazolyl)ethyl]-4-(3-pyridyl)-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. Further, the 3-pyridyl group at the 4-position was shown to be necessary for *in vivo* thromboxane A_2 synthetase-inhibitory activity.

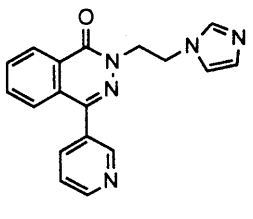
Keywords phthalazinone; TXA_2 synthetase-inhibitory activity; bronchodilatory activity; antiasthmatic agent

Previous studies in this series have demonstrated that 2-[2-(1-imidazolyl)ethyl]-4-(3-pyridyl)-1(2*H*)-phthalazinone (**1**) has both unexpectedly high thromboxane A_2 (TXA_2) synthetase-inhibitory and bronchodilatory activities *in vivo* in spite of lacking both activities *in vitro*, and that 4-(5-ethyl-2-thienyl)-2-[2-(1-imidazolyl)ethyl]-1(2*H*)-phthalazinone (**2**) is one of the most potent agents known, with well-rounded dual activities.[2] We have further examined the effects of the 2- and 4-substituents on the respective activities of **1**, and it was disclosed firstly that the 3-pyridyl group plays a critical role in TXA_2 synthetase inhibition and secondly that the hydrophobicity of **1** exerts a marked influence on bronchodilatory activity.[3] As for the pharmacological activities of **2**, it has been shown that the phenyl moiety of the phthalazinone skeleton plays an important role in both activities[1]; this aspect has been not examined yet with **1**. In a continuation of our studies on the structure–activity relationship of **1**, we undertook the synthesis and pharmacological evaluation of several previously unreported compounds related to **1**. This paper describes the synthesis and pharmacological evaluation of phthalazinone derivatives having modified pyridyl rings and substituted phenyl rings as compounds with both TXA_2 synthetase-inhibitory and bronchodilatory activities.

4-(6-Ethyl-3-pyridyl)-2-[2-(1-imidazolyl)ethyl]-1(2*H*)-phthalazinone (**6**) was synthesized using 2-(6-ethylnicotinoyl)benzoic acid (**4**) as the key intermediate in the same way as described in a previous paper.[1] Preparation of **4** was performed by the reaction of phthalic anhydride (**3**) with 2-ethylpyridine-5-magnesium bromide (derived from 5-bromo-2-ethylpyridine *via* the 5-lithiopyridine). Cyclization of **4** using hydrazine hydrate to the phthalazinone (**5**) followed by alkylation with 1-(2-bromoethyl)imidazole in the presence of potassium carbonate gave **6** (Chart 1).

The methylene-inserted analogue of **1** (**8**) was prepared from 4-(3-pyridyl)methyl-1(2*H*)-phthalazinone (**7**), which was obtained according to the known procedure[4] involving decarboxylative condensation of **3** with 3-pyridylacetic acid and subsequent treatment with hydrazine hydrate (Chart 1).

6-(3-Pyridyl)-3(2*H*)-pyridazinone (**9**), necessary for the synthesis of the pyridazinone analogue (**10**), was prepared by the known procedure[5] from 3-acetylpyridine, involving condensation with glyoxylic acid and subsequent cycliza-



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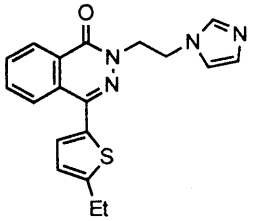

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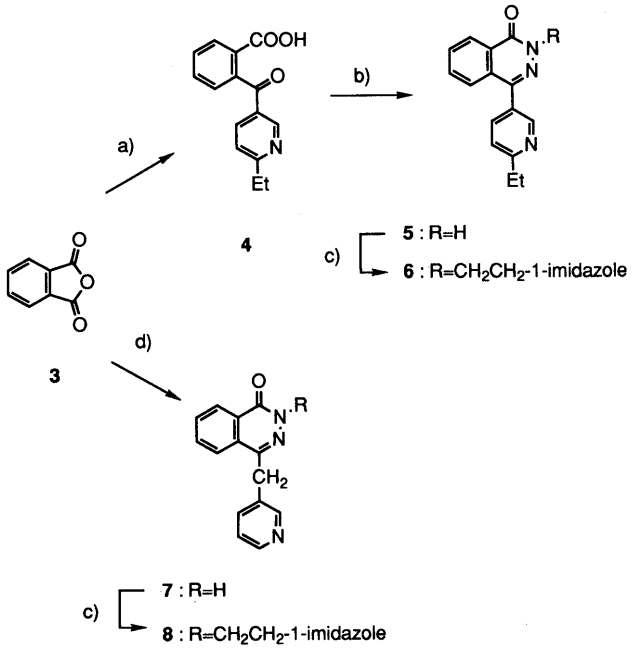
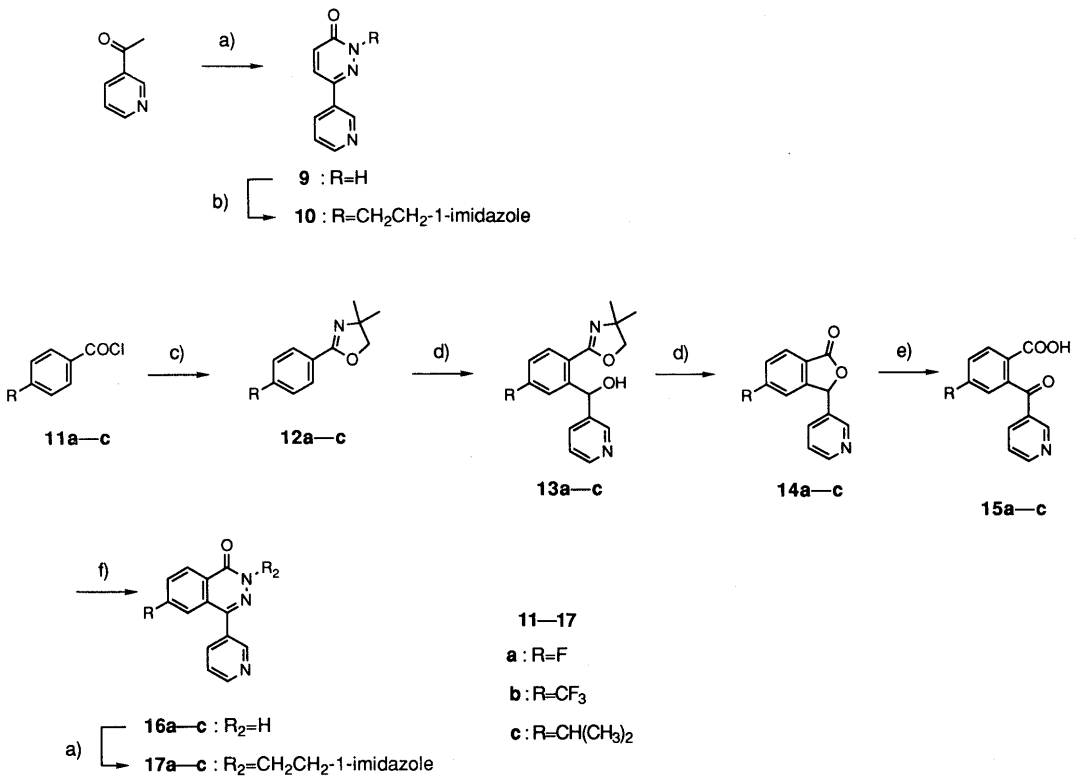
Fig. 1. 4-(3-Pyridyl)- and 4-(5-Ethyl-2-thienyl)-2-[2-(1-imidazolyl)-ethyl]-1(2*H*)-phthalazinones



a) 2-ethylpyridine-5-magnesium bromide, 0 °C; b) H_2NNH_2, EtOH, reflux; c) 1-(2-bromoethyl)imidazole, K_2CO_3, 80 °C; d) (i) 3-pyridineacetic acid, AcONa, 200 °C; (ii) H_2NNH_2, EtOH, reflux.

Chart 1



a) (i) $OHCCO_2H$, K_2CO_3, H_2O; (ii) AcOH (iii) H_2NNH_2, reflux; (b) 1-(2-bromoethyl)imidazole, K_2CO_3, 80 °C; c) (i) 2-amino-2-methyl-1-propanol; (ii) $SOCl_2$; d) *n*-BuLi, 3-pyridinecarboxaldehyde, −78 °C; e) HCl aq., reflux; f) $KMnO_4$, 60 °C; g) H_2NNH_2, EtOH, reflux.

Chart 2

tion/dehydration after acidification: compound **10** was obtained by imidazolylethylation of **9** (Chart 2).

In order to obtain 6-substituted derivatives of **1** (**17a—c**), the corresponding 4-substituted 2-nicotinoylbenzoic acids (**15a—c**) were prepared as follows. The 4-substituted benzoyl chlorides (**11a—c**) were converted to the oxazoline derivatives (**12a—c**) by the known method[6] and their *o*-lithiated derivatives were treated with 3-pyridinecarboxaldehyde to give the condensation products (**13a—c**), which underwent hydrolysis of the oxazolinyl group and cyclization upon heating with 4 N hydrochloric acid to produce the 5-substituted 3-(3-pyridyl)phthalides (**14a—c**). Alkaline hydrolysis of **14** followed by potassium permanganate oxidation afforded the desired **15a—c**. Cyclization of **15a—c** with hydrazine hydrate to the phthalazinones (**16a—c**) and imidazolylethylation of **16a—c** led to the 6-substituted phthalazinones (**17a—c**) (Chart 2).

In order to test the TXA_2 synthetase inhibitory-activity, an *in vitro* assay with TXA_2 synthetase from rabbit was employed, together with measurement of TXA_2 production in rat serum as an *in vivo* assay. None of these compounds inhibited prostaglandin I_2 (PGI_2) formation, which is consistent with a mechanism of selective TXA_2 synthetase inhibition.[7] To evaluate bronchodilatory activity, we measured spontaneous tone inhibition with guinea pig tracheal strips as an *in vitro* assay and the inhibitory effect on histamine-induced bronchoconstriction using anesthetized guinea pigs as an *in vivo* assay. We used OKY-046[8] for TXA_2 synthetase inhibition and aminophylline for bronchodilation as active controls.

TABLE I. TXA_2 Synthetase-Inhibitory and Bronchodilatory Activities

Compound	Percent 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] Percent inhibition
1[d]	38	90	4.57	97
6	55	28	4.71	85
8	12	76	4.37	51
10	20	25	<4.0	0
17a	53	62	4.15	74
17b	7		4.43	67
17c	23	0	4.96	11
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 8.

4-(6-Ethyl-3-pyridyl)-2-[2-(1-imidazolyl)ethyl]-1(2*H*)-phthalazinone (**6**) exhibited a higher *in vitro* TXA_2 synthetase-inhibitory activity than the parent compound **1**, but the *in vivo* TXA_2 synthetase-inhibitory activity was reduced (Table I). On the other hand, the methylene-inserted compound **8** showed a much lower *in vitro* TXA_2 synthetase-inhibitory activity and a more-or-less retained *in vivo* activity. These observations seem to indicate that

the *in vivo* TXA_2 synthetase-inhibitory activity is mainly derived from the pyridyl group at the 4-position rather than the 2-imidazolylethyl group. The reduced *in vivo* activity of **6** is consistent with the view that introduction of substituents into the pyridine ring generally reduces the TXA_2 synthetase-inhibitory activity due to a steric effect.[9] The retained potency of the methylene-inserted compound **8** in the *in vivo* TXA_2 synthetase inhibition may be ascribed to the 3-pyridyl group in the 4-substituent, in spite of the fact that compounds having a methylene-inserted phenyl group at the 4-position were much less effective *in vitro* than the corresponding compounds with a directly bound phenyl group.[2]

The pyridazinone derivative **10** exhibited no significant TXA_2 synthetase-inhibitory activity or bronchodilatory activity; removal of the phenyl moiety substantially eliminates both activities, providing additional evidence for the critical role of the phenyl moiety in both activities.

Among the 6-substituted phthalazinones (**17a—c**), only the 6-fluoro derivative (**17a**) showed a retained TXA_2 synthetase-inhibitory activity, while the other two compounds having bulkier substituents (**17b**, 6-CF_3 and **17c**, 6-iso-Pr) had no significant activity, probably due to steric effects (Table I).

With respect to the bronchodilatory activity, except for compound **10**, all of the tested compounds retained the activity to some degree. As previously demonstrated, the bronchodilatory activity of the phthalazinone series is markedly affected by hydrophobicity.[2] Thus, the much decreased activity of **10** might be ascribed to the decrease in hydrophobicity caused by loss of the phenyl moiety, while the retained activities of the phthalazinone derivatives (**6**, **8**, **17a—c**) seem to be a reflection of the general effectiveness of the phenyl moiety in the phthalazinone skeleton for bronchodilation.

In conclusion, the 3-pyridyl group at the 4-position and the phenyl moiety of the phthalazinone skeleton in **1** appear to be necessary for both TXA_2 synthetase-inhibitory and bronchodilatory activities. Further, the steric effect of a substituent on the phenyl moiety markedly affects TXA_2 synthetase-inhibitory activity.

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 270-30 infrared spectrometer. The ^{1}H-NMR spectra were taken with a Hitachi R-24B high-resolution magnetic resonance spectrometer (60 MHz) using tetramethylsilane as an internal standard. Mass spectra (MS) were obtained on a Shimadzu 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.

4-(6-Ethyl-3-pyridyl)-2-[2-(1-imidazolyl)ethyl]-1(2*H*)-phthalazinone (6) 2-Ethyl-5-bromopyridine was prepared from 2,5-dibromopyridine according to the known procedure.[10] A solution of 2-ethyl-5-bromopyridine (9.3 g, 50 mmol) in ether (75 ml) was added dropwise to a solution of 1.6 M *n*-BuLi (in 38 ml hexane, 60 mmol) in ether (150 ml) at −78 °C under a nitrogen atmosphere, and the mixture was stirred for 30 min at the same temperature. To this mixture, a solution prepared from magnesium (2.4 g, 60 mmol) and 1,2-dibromoethane (8.6 ml, 100 mmol) in dry THF (500 ml) was added at 0 °C. The solution was added dropwise to a solution of phthalic anhydride (**3**) (7.4 g, 50 mmol) in dry THF (150 ml) at 0 °C and the mixture was stirred for 1 h at the same temperature. Aqueous NH_4Cl was added, the whole was adjusted to pH 5 and the organic layer was separated. The extract was washed with brine, dried, and concentrated under reduced pressure. The residual oil was chromatographed on silica gel with $CHCl_3$–MeOH (40 : 1) to give 5.8 g (45%) of 2-(6-ethylnicotinoyl)benzoic acid (**4**) as white crystals, mp 162—164 °C ($CHCl_3$–EtOH). IR (KBr): 3450, 1695, 1665 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.20 (3H, t, J=7 Hz), 2.78 (2H, q, J=7 Hz), 7.10—8.26 (6H, m), 8.72 (1H, d, J=2 Hz), 12.75 (1H, br s). A solution of **4** (2.5 g, 12 mmol) and 80% hydrazine hydrate (2.3 g, 36 mmol) in EtOH (100 ml) was refluxed for 2 h. After cooling, the precipitates were collected, washed with EtOH, and dried under reduced pressure to give 2.2 g (73%) of 4-(6-ethyl-3-pyridyl)-1(2*H*)-phthalazinone (**5**) as white crystals, mp 217—219 °C (EtOH). IR: 1660 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.38 (3H, t, J=7 Hz), 2.95 (2H, q, J=7 Hz), 7.65—7.93 (5H, m), 8.42—8.63 (1H, m), 8.76 (1H, d, J=2 Hz), 11.53 (1H, br s). MS *m/z*: 251 (M^+, 100). *Anal.* Calcd for $C_{15}H_{13}N_3O$: C, 71.70; H, 5.22; N, 16.72. Found: C, 71.78; H, 5.12; N, 16.69. A mixture of **5** (2 g, 8 mmol), 1-(2-bromoethyl)imidazole hydrogen bromide (2.4 g, 9.6 mmol), and K_2CO_3 (4.3 g, 32 mmol) in DMF (50 ml) was stirred for 3 h at 70 °C. It was then cooled, and 2 N HCl and AcOEt were added. 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 1.8 g (65%) of **6** as white crystals, mp 127—129 °C ($CHCl_3$–hexane). IR (KBr): 1660 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.37 (3H, t, J=7 Hz), 2.93 (2H, q, J=7 Hz), 4.40—4.76 (4H, m), 6.88—7.92 (8H, m), 8.35—8.74 (2H, m). MS *m/z*: 345 (M^+, 21), 95 (100). *Anal.* Calcd for $C_{20}H_{19}N_5O \cdot H_2O$: C, 66.10; H, 5.82; N, 19.27. Found: C, 66.26; H, 5.80; N, 19.29.

2-[2-(1-Imidazolyl)ethyl]-4-(3-pyridylmethyl)-1(2*H*)-phthalazinone (8) 4-(3-Pyridyl)methyl-1(2*H*)-phthalazinone[11] (**7**) was prepared from phthalic anhydride (**3**) according to the known procedure.[4] Introduction of a 2-(1-imidazolyl)ethyl group was performed as described above and recrystallization from $CHCl_3$–hexane gave **8** (83%) as white crystals, mp 113—115 °C ($CHCl_3$–hexane). IR (KBr): 1655 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 4.27 (2H, s), 4.38—4.65 (4H, m), 6.86—7.07 (2H, m), 7.20—7.48 (3H, m), 7.62—7.87 (3H, m), 8.32—8.63 (3H, m). *Anal.* Calcd for $C_{19}H_{17}N_5O$: C, 68.87; H, 5.17; N, 21.13. Found: C, 68.82; H, 5.30; N, 21.24.

2-[2-(1-Imidazolyl)ethyl]-6-(3-pyridyl)-3(2*H*)-pyridazinone (10) 6-(3-Pyridyl)-3(2*H*)-pyridazinone (**9**) was prepared from 3-acetylpyridine according to the known procedure.[5] Treatment of **9** with 1-(2-bromoethyl)imidazole by the method described above gave **10** (74%) as white crystals, mp 104—106 °C ($CHCl_3$–hexane). IR (KBr): 1680 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 4.45—4.72 (2H, m), 6.90—7.18 (3H, m), 7.23—8.05 (4H, m), 8.57—8.96 (2H, m). *Anal.* Calcd for $C_{14}H_{13}N_5O \cdot 2/3H_2O$: C, 60.21; H, 5.17; N, 25.08. Found: C, 60.05; H, 5.00; N, 25.05.

6-Fluoro-2-[2-(1-imidazolyl)ethyl]-4-(3-pyridyl)-1(2*H*)-phthalazinone (17a) 2-(4-Fluorophenyl)-4,4-dimethyloxazoline[12] (**12a**) was prepared according to the known procedure from 4-fluorobenzoyl chloride (**11a**).[6] A solution of 1.5 M *n*-BuLi (in 6.5 ml hexane, 9.7 mmol) was added dropwise to a solution of **12a** (1.7 g, 8.8 mmol) in ether (15 ml) at −78 °C under a nitrogen atmosphere and the whole stirred for 1 h at the same temperature. A solution of 3-pyridinecarboxaldehyde (1 g, 9.3 mmol) in ether (10 ml) was poured into the reaction mixture in one portion at −78 °C under nitrogen and the mixture stirred for 1.5 h at the same temperature. The mixture was poured into water (100 ml) and extracted with AcOEt. The extract was dried, concentrated, and chromatographed on silica gel with $CHCl_3$–acetone 20 : 1 to give 2.3 g of **13a** (87%) as a pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ: 1.02 (3H, s), 1.33 (3H, s), 3.94 (2H, s), 5.85—5.92 (1H, m), 6.68—7.27 (2H, m), 7.43—8.07 (3H, m), 8.30—8.53 (2H, m). MS *m/z*: 301 (M^++1, 37), 229 (100). A mixture of 2-[4-fluoro-2-hydroxy-(3-pyridyl)methylphenyl]oxazoline (**13a**) (2.3 g, 7.7 mmol) and 4 N HCl (45 ml) was refluxed for 1 h. After cooling, the mixture was adjusted to pH 6 with saturated $NaHCO_3$, and extracted with AcOEt. The extract was dried and concentrated under reduced pressure to give 1.4 g (79%) of 5-fluoro-3-(3-pyridyl)phthalide (**14a**) as a white solid. IR (KBr): 1760 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 6.35 (1H, s), 6.80—7.54 (4H, m), 7.82—8.03 (1H, m), 8.40—8.68 (2H, m). MS *m/z*: 229 (M^+, 13), 124 (100). Powdered $KMnO_4$ (1.4 g, 9 mmol) was added in portions to a mixture of **14a** (1.4 g, 6 mmol), pyridine (12 ml) and 25% aqueous KOH (24 ml) at 60 °C, and the whole was stirred for 5 h at the same temperature. After cooling, the mixture was filtered, and the resulting filtrate was adjusted to pH 5 and extracted with $CHCl_3$. The extract was dried and concentrated under reduced pressure to give 0.85 g (58%) of 4-fluoro-2-nicotinoylbenzoic acid (**15a**) as a pale yellow

solid, mp 208—210 °C. IR (KBr): 3450, 1700, 1675 cm^{-1}. ^{1}H-NMR (CD_3OD) δ: 7.02—7.68 (3H, m), 7.90—8.28 (2H, m), 8.44—8.83 (2H, m). MS *m/z*: 245 (M^+, 3), 107 (100). *Anal.* Calcd for $C_{13}H_8FNO_3$: C, 63.68; H, 3.29; N, 5.71. Found: C, 63.50; H, 3.15; N, 5.58. In a similar manner to that described above, treatment of **15a** with hydrazine hydrate afforded 6-fluoro-4-(3-pyridyl)-1(2*H*)-phthalazinone (**16a**) (81%) as white crystals, mp 260—262 °C (EtOH). IR (KBr) 1680 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 7.15—7.73 (3H, m), 7.82—8.11 (1H, m), 8.40—8.87 (3H, m), 12.50 (1H, br s). MS *m/z*: 242 (M^++1, 100), 241 (M^+, 73). *Anal.* Calcd for $C_{13}H_8FN_3O$: C, 64.73; H, 3.34; N, 17.42. Found: C, 64.71; H, 3.19; N, 17.31. Imidazolylethylation gave **17a** (52%) as white crystals, mp 142—143 °C ($CHCl_3$–hexane). IR (KBr): 1650 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 4.31—4.80 (4H, m), 6.85—7.84 (7H, m), 8.33—8.82 (3H, m). MS *m/z*: 335 (M^+, 17), 96 (100). *Anal.* Calcd for $C_{18}H_{14}FN_5O \cdot 1/4H_2O$: C, 63.62; H, 4.30; N, 20.61. Found: C, 63.65; H, 4.47; N, 20.40.

In a similar manner, **12—17b** and **12—17c** were prepared. 4,4-Dimethyl-2-(4-trifluoromethylphenyl)oxazoline[13] (**12b**) was prepared according to the known procedure.[6] 4,4-Dimethyl-2-(4-isopropylphenyl)oxazoline (**12c**) was obtained as an oil, yield 39%, 135—140 °C (8 mmHg). ^{1}H-NMR ($CDCl_3$) δ: 1.26 (6H, d, J=7 Hz), 1.33 (6H, s), 2.21 (1H, hept, J=7 Hz), 4.03 (2H, s), 7.22 (2H, d, J=6 Hz), 7.81 (2H, d, J=6 Hz). **13b** as an oil, yield 78%. ^{1}H-NMR ($CDCl_3$) δ: 1.01 (3H, s), 1.32 (3H, s), 4.01 (2H, s), 6.09 (1H, br s), 7.05—7.35 (2H, m), 7.48—8.13 (4H, m), 8.36—8.67 (2H, m). MS *m/z*: 350 (M^+, 7), 279 (100). **13c** as an oil, yield 19%. ^{1}H-NMR ($CDCl_3$) δ: 0.93 (3H, s), 1.23 (6H, d, J=7 Hz), 1.30 (3H, s), 2.91 (1H, hept, J=7 Hz), 3.90 (2H, s), 5.90 (1H, br s), 6.98—7.33 (3H, m), 7.45—7.87 (2H, m), 8.02—8.53 (3H, m). MS *m/z*: 324 (M^+, 21), 253 (100). **14b** as an oil, yield 88%. ^{1}H-NMR ($CDCl_3$) δ: 6.54 (1H, s), 7.22—8.30 (5H, m), 8.64—8.82 (2H, m). MS *m/z*: 279 (M^+, 14), 106 (100). **14c** as an oil, yield 82%. ^{1}H-NMR ($CDCl_3$) δ: 1.24 (6H, d, J=7 Hz), 2.96 (1H, hept, J=7 Hz), 6.36 (1H, s), 7.05—7.92 (5H, m), 8.48—8.68 (2H, m). MS *m/z*: 253 (M^+, 26), 227 (100). **15b** as a white solid, yield 36%, mp 232—235 °C. IR (KBr): 3450, 1680 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 7.30—7.65 (1H, m), 7.70—8.34 (5H, m), 8.52—8.86 (2H, m). *Anal.* Calcd for $C_{14}H_8F_3NO_3$: C, 56.96; H, 2.73; N, 4.74. Found: C, 56.82; H, 2.82; N, 4.68. **15c** as a crude solid, yield 45%. MS *m/z*: 269 (M^+, 14), 192 (100). **16b** as a white solid, yield 80%, mp 246—248 °C. IR (KBr): 1680 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 7.45—7.88 (2H, m), 7.98—8.32 (2H, m), 8.45—8.89 (3H, m), 12.03 (1H, br s). MS *m/z*: 292 (M^++1, 100), 291 (M^+, 38). *Anal.* Calcd for $C_{14}H_8F_3N_3O$: C, 57.74; H, 2.77; N, 14.43. Found: C, 57.55; H, 2.83; N, 14.40. **16c** as a crude solid, yield 57%. ^{1}H-NMR ($CDCl_3$) δ: 1.27 (6H, d, J=7 Hz), 3.07 (1H, hept, J=7 Hz), 7.25—8.08 (4H, m), 8.40—8.95 (3H, m), 11.90 (1H, br s). MS *m/z*: 265 (M^+, 33), 223 (100). **17b** as white crystals, yield 66%, mp 150—151 °C ($CHCl_3$–hexane). IR (KBr): 1655 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 4.28—4.79 (4H, m), 6.82—7.02 (2H, m), 7.20—8.13 (5H, m), 8.47—8.80 (3H, m). MS *m/z*: 385 (M^+, 26), 95 (100). *Anal.* Calcd for $C_{19}H_{14}F_3N_5O$: C, 59.22; H, 3.66; N, 18.17. Found: C, 59.29; H, 3.87; N, 18.11. **17c** as white crystals, yield 58%, mp 151—152 °C ($CHCl_3$–hexane). IR (KBr): 1645 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.28 (6H, d, J=7 Hz), 3.05 (1H, hept, J=7 Hz), 4.40—4.83 (4H, m), 6.98—7.14 (2H, m), 7.35—7.98 (5H, m), 8.52 (1H, d, J=8 Hz), 8.75—8.94 (2H, m). MS *m/z*: 359 (M^+, 14), 95 (100). *Anal.* Calcd for $C_{21}H_{21}N_5O$: C, 70.18; H, 5.89; N, 19.48. Found: C, 69.96; H, 5.94; N, 19.23.

In Vitro* Enzyme Assay of TXA_2 Synthetase** Rabbit platelet microsomes as the enzyme source were prepared according to the methods of Needleman *et al.*[14] 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 adding arachidonic acid (1—3 μM), the reaction mixture was incubated for 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 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 above. The percent inhibition of TXA_2 synthetase was calculated as 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 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.

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 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 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) a 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 artificially ventilated (10 ml/kg, 60 strokes/min). The pressure in the respirator system, *i.e.* the insufflation pressure, was measured constantly 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. 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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References

1) M. Yamaguchi, T. Koga, K. Kamei, M. Akima, A. Maruyama, T. Kuroki, M. Hamana, N. Ohi: Novel Antiasthmatic Agents with Dual Activities of Thromboxane A_2 Synthetase Inhibition and Bronchodilation. III. 4-[2-(5-Ethylthienyl)]-2-[2-(1-imidazolyl)ethyl]-1(2*H*)-phthalazinones. *Chem. Pharm. Bull.*, **42**, 1601 (1994).
2) M. Yamaguchi, K. Kamei, T. Koga, M. Akima, T. Kuroki, N. Ohi, *J. Med. Chem.*, **36**, 4052 (1993).
3) M. Yamaguchi, K. Kamei, T. Koga, M. Akima, A. Maruyama, T. Kuroki, N. Ohi, *J. Med. Chem.*, **36**, 4061 (1993).
4) R. Weiss, *Org. Synth.*, **13**, 10 (1933).
5) W. J. Coates, A. McKillop, *Synthesis*, **1993**, 334.
6) A. I. Meyers, D. L. Temple, D. Haidukewych, E. D. Mihelich, *J. Org. Chem.*, **18**, 2787 (1974).
7) W. B. Jr. Wright, J. B. Press, P. S. Chan, J. W. Marsico, M. F. Hang, J. Lucas, J. Tauber, A. S. Tomcufcik, *J. Med. Chem.*, **29**, 523 (1986).
8) K. Iizuka, K. Akahane, D. Momose, M. Nakazawa, T. Tanouchi, M. Kawamura, I. Ohyama, I. Kajiwara, Y. Iguchi, T. Okada, K. Taniguchi, T. Miyamoto, M. Hayashi, *J. Med. Chem.*, **24**, 1139 (1981).
9) K. Kato, S. Ohkawa, S. Terao, Z. Terashita, K. Nishikawa, *J. Med. Chem.*, **28**, 287 (1985).
10) J. W. Tilley, S. Zawoiski, *J. Org. Chem.*, **53**, 386 (1988).
11) K. Uenishi, K. Kosegi, Y. Asaumi, Y. Ishizuka, H. Yaginuma, Japan. Patent 03106875 (1991) [*Chem. Abstr.*, **115**, 256197*s* (1991)].
12) M. S. Newman, R. Kannan, *J. Org. Chem.*, **44**, 3388 (1979)
13) M. S. Newman, S. Veeraraghavan, *J. Org. Chem.*, **48**, 3246 (1983).
14) P. Needleman, S. Moncada, S. Bunting, J. R. Vane, M. Hamberg, B. Samuelsson, *Nature* (London), **261**, 558 (1976).