Novel Antiasthmatic Agents with Dual Activities of Thromboxane A_2 Synthetase Inhibition and Bronchodilation. 2. 4-(3-Pyridyl)-1(2*H*)-phthalazinones

Masahisa Yamaguchi,* Kenshi Kamei, Takaki Koga, Michitaka Akima, Akinori Maruyama, Toshio Kuroki, and Nobuhiro Ohi

Fuji-gotemba Research Laboratories, Chugai Pharmaceutical Company, Ltd., 135, Komakado 1-Chome, Gotemba-shi, Shizuoka, 412, Japan

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A series of novel 4-(3-pyridyl)-1(2*H*)-phthalazinone derivatives which possess dual activities of thromboxane A_2 (TXA_2) synthetase inhibition and bronchodilation was synthesized, and their pharmacological activities were evaluated. While the length and the bulk of 2-alkyl substituents had no influence on either activity, the 2-substituents with polar groups reduced bronchodilatory activity. Furthermore, we introduced heteroaromatic nuclei into the 4-position of the phthalazinone and found that 1-imidazolyl (**13a**) and 5-thiazolyl (**16b** and **16c**) derivatives were as active as the parent 3-pyridyl compound **5b**. These findings suggest that heteroaromatic nuclei at the 4-position of phthalazinones play a critical role in TXA_2 synthetase inhibition. Additionally, the hydrophobicity of the compounds was found to exert a marked influence on bronchodilatory activity. These observations led to the selection of 2-ethyl-4-(3-pyridyl)-1(2*H*)-phthalazinone (**5b**) (KK-505) and 2-methyl-4-(5-thiazolyl)-1(2*H*)-phthalazinone (**16b**) (KK-562) for further studies. Although their precise mechanism of action remains unclear, this series of novel phthalazinone derivatives represents a new class of antiasthma agents with dual activities.

In the preceding paper,[1] we described the synthesis and pharmacological evaluation of 4-substituted 2-[2-(1-imidazolyl)alkyl]-1(2*H*)-phthalazinones and showed that certain of these compounds have well-rounded activities with respect to thromboxane A_2 (TXA_2) synthetase inhibition and bronchodilation. Among these compounds, 2-[2-(1-imidazolyl)ethyl]-4-(3-pyridyl)-1(2*H*)-phthalazinone (**1**) was particularly of interest, because **1** was found to have unexpectedly high activities *in vivo* in spite of no significant activities *in vitro*.

Since the 2-substituent of **1** is considered to readily undergo metabolic conversion, it may be assumed that the actual species exhibiting *in vivo* activities are the metabolites of **1**, that is, the desimidazolyl or desimidazolylethyl compound (Figure 1). In fact, we identified 4-(3-pyridyl)-1(2*H*)-phthalazinone as the main metabolite of 2-substituted 4-(3-pyridyl)-1(2*H*)-phthalazinone derivatives in further study. In order to explore this, we prepared 4-(3-pyridyl)phthalazinones bearing various types of substituents at the 2-position and carried out pharmacological evaluation of these compounds including 4-(3-pyridyl)phthalazinone (**4**).

The 3-pyridyl moiety has been reported to be the component effective for TXA_2 synthetase inhibitory activity as seen for the 1-imidazolyl group.[2] Taking into account this fact, we also undertook the synthesis and pharmacological evaluation of 2-alkyl-1(2*H*)-phthalazinone derivatives bearing several heteroaromatic nuclei other than 3-pyridyl at the 4-position.

We report novel antiasthmatic agents with novel structural features resulting in dual TXA_2 synthetase inhibition and bronchodilation.

Chemistry

3-Pyridyl-1(2*H*)-phthalazinone (**4**) was prepared by a reaction sequence described in a preceding paper,[1] and a variety of substituents were introduced into the 2-position

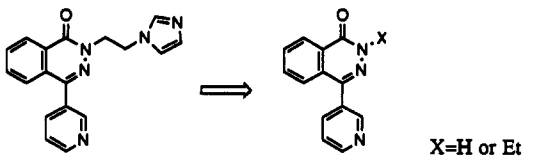

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Figure 1.

of **4** in the usual manner. Treatment of **4** with halogenoalkanes and acyl halides in the presence of sodium hydride (NaH) gave the corresponding 2-alkyl (**5a–r** and **5u**) and 2-acyl derivatives (**5s** and **5t**), respectively, in generally good yields (Scheme I). Some ω-substituted alkyl derivatives (**7a–e**) were prepared through (ω-bromoalkyl)-phthalazinones (**6a** and **b**) (Scheme II), and 2-hydroxyethyl (**8a**) and ω-carboxyalkyl derivatives (**8b–d**) were obtained by alkaline hydrolysis of the corresponding esters (**5o–r**) (Scheme III). 2-(2-Phthalimidoethyl)phthalazinone (**5u**) reacted with hydrazine to give the 2-aminoethyl derivative (**9**), which was transformed into the sulfonyl amide (**10**) by reaction with benzenesulfonyl chloride and NaH (Scheme IV). Treatment of the 2-(3-carboxypropyl)-phthalazinone (**8c**) with phosphorus pentachloride and triethylamine, followed by reaction with isopropylamine, provided the isopropylamide (**11**).

Further, several analogous phthalazinones having heteroaryl substituents other than the 3-pyridyl group at the 4-position were prepared. 4-(1-Imidazolyl)- and 4-(1-triazolyl)phthalazinones (**12a** and **12b**) were obtained by treatment of 1,4-dichlorophthalazine with imidazole and 1,2,4-triazole in the presence of NaH, respectively, followed by alkaline hydrolysis of the 1-chloro substituents, and **12a** and **12b** were transformed into 2-ethyl derivatives (**13a** and **13b**) by treatment with ethyl bromide–NaH (Scheme V).

For the synthesis of the 2-thiazolyl, 5-thiazolyl, 2-pyrazinyl, and 5-pyrimidyl analogues, we chose the corresponding 2-heteroaroylbenzoic acids (**14a–d**) as the key intermediates. 2-(2-Thiazolyl)benzoic acid (**14a**) was obtained by

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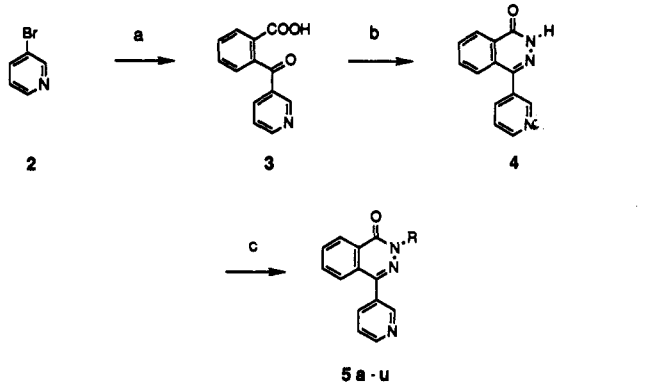
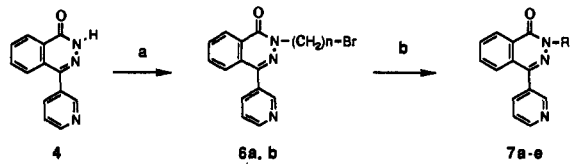
Scheme I[a]



5a	R=Me	5k	R=benzyl
b	R=Et	l	R=3-chlorobenzyl
c	R=n-propyl	m	R=CH_2COCH_3
d	R=i-propyl	n	R=$(CH_2)_2OMe$
e	R=n-butyl	o	R=$(CH_2)_2OAc$
f	R=n-octyl	p	R=CH_2COOEt
g	R=2-propenyl	q	R=$(CH_2)_3COOEt$
h	R=3-methyl-2-butenyl	r	R=$CH_2C_6H_4$-4-COOMe
i	R=cyclopentyl	s	R=CO-2-thienyl
j	R=cyclohexylmethyl	t	R=$SO_2C_6H_5$
		u	R=$(CH_2)_2$-phthalimide

[a](a) (i) *n*-BuLi, −78 °C; (ii) phthalic anhydride; (b) H_2NNH_2, Δ; (c) (i) NaH; (ii) R-halogen.

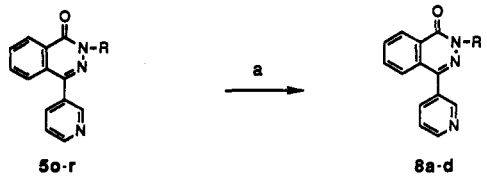
Scheme II[a]



6a	n=2	7a	R=$(CH_2)_2$-1-tetrazole
b	n=4	b	R=$(CH_2)_2$-SPh
		c	R=$(CH_2)_2$-S-2-thiazoline
		d	R=$(CH_2)_2$-S-2-pyrimidine
		e	R=$(CH_2)_4$-S-2-pyrimidine

[a](a) (i) NaH; (ii) $Br(CH_2)_nBr$; (b) RH or K, NaH.

Scheme III[a]

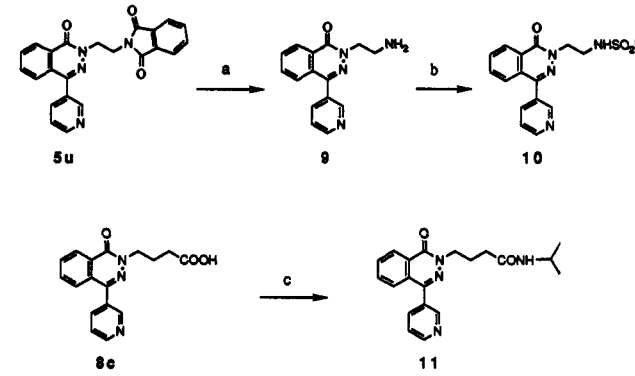


5o	R=$(CH_2)_2OAc$	8a	R=$(CH_2)_2OH$
p	R=CH_2COOEt	b	R=CH_2COOH
q	R=$(CH_2)_3COOEt$	c	R=$(CH_2)_3COOH$
r	R=$CH_2C_6H_4$-4-COOMe	d	R=$CH_2C_6H_4$-4-COOH

[a](a) NaOH(aq).

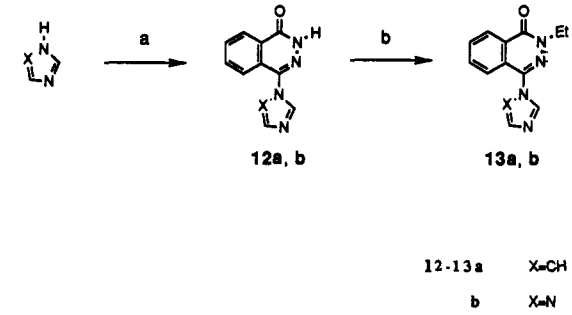
reaction of the phthalic anhydride with 2-thiazolyllithium prepared from thiazole and *n*-butyllithium (*n*-BuLi) at −78 °C. Preparation of the 5-thiazolyl analogue (**14b**) was effected by a similar process using 2-(trimethylsilyl)-

Scheme IV[a]



[a](a) H_2NNH_2, Δ, 87% yield; (b) (i) NaH; (ii) $ClSO_2Ph$, 45% yield; (c) (i) PCl_5, Et_3N; (ii) isopropylamine, 12% yield.

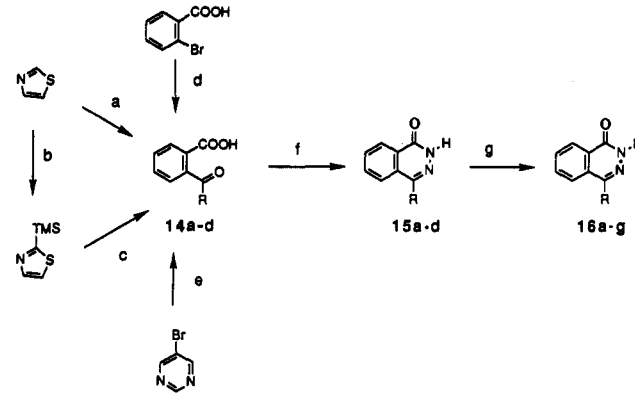
Scheme V[a]



12-13a	X=CH		
b	X=N		

[a](a) (i) NaH, 1,4-dichlorophthalazine; (ii) NaOH aq; (b) (i) NaH; (ii) ethyl bromide.

Scheme VI[a]

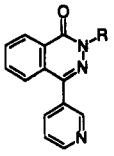


14-15a	R=2-thiazolyl	16a	R=2-thiazolyl	R_2=Et
b	R=5-thiazolyl	b	R=5-thiazolyl	R_2=Me
c	R=2-pyrazinyl	c	R=5-thiazolyl	R_2=Et
d	R=5-pyrimidyl	d	R=5-thiazolyl	R_2=i-Propyl
		e	R=5-thiazolyl	R_2=i-Butyl
		f	R=2-pyrazinyl	R_2=Et
		g	R=5-pyrimidyl	R_2=Et

[a](a) (i) *n*-BuLi, −78 °C; (ii) phthalic anhydride; (b) (i) *n*-BuLi, −78 °C; (ii) TMSCl; (c) (i) *n*-BuLi, −78 °C; (ii) phthalic anhydride; (ii) H^+; (d) (i) 2 equiv of *n*-BuLi, −78 °C; (ii) 2-(methoxycarbonyl)pyrazine; (e) (i) *n*-BuLi, −110 °C; (ii) phthalic anhydride; (f) H_2NNH_2, Δ; (g) (i) NaH; (ii) R_2Br.

thiazole[3] instead of thiazole, followed by acidic hydrolysis of the trimethylsilyl group. Because of the poor availability of 2-bromopyrazine, the 2-pyrazinyl derivative (**14c**) was prepared from (2-carboxyphenyl)lithium and 2-(methoxycarbonyl)pyrazine.[4] Thus, successive treatment of 2-bromobenzoic acid with 2 equiv of *n*-BuLi and excess

Table I. 4-(3-Pyridyl)-1(2*H*)-phthalazinones



no.	R	yield[a] (%)	mp, °C (recrystn solvent)[b]	% inhibn of TXA_2 prodn *in vitro* at 1 μM	% inhibn of TXA_2 prodn *ex vivo*[c] 30 mg/kg po	bronchodilatory activity *in vitro*[d] −log[IC_{50}(M)]	bronchodilatory activity *in vivo*[e] % inhibn	formula[f]
1	$(CH_2)_2$-1-imidazole[g]			37	90	4.57	97	
4	H	90	269–270 (A)	26	78	4.99		$C_{16}H_8N_3O$
5a	CH_3	84	153–154 (B)	63	94	4.94	97	$C_{14}H_{11}N_3O$
5b	C_2H_5	91	156–157 (C)	87	92	5.88	100	$C_{15}H_{13}N_3O$
5c	$(CH_2)_2CH_3$	84	142–143 (C)	86	93	5.50		$C_{16}H_{15}N_3O$
5d	$CH(CH_3)_2$	72	126–127[h] (C)	87	82	5.51		$C_{16}H_{15}N_3O$
5e	$(CH_2)_3CH_3$	66	115–116 (C)	91	100	5.64		$C_{17}H_{17}N_3O$
5f	$(CH_2)_7CH_3$	66	59–60 (C)	99		4.66		$C_{21}H_{25}N_3O$
5g	$CH_2CH{=}CH_2$	70	145–146 (C)	70	82	5.67	100	$C_{16}H_{13}N_3O$
5h	$CH_2CH{=}C(CH_3)_2$	71	152–153[h] (C)	94		5.86		$C_{16}H_{17}N_3O$
5i	C_5H_9	66	135–137 (C)	97	80	5.56		$C_{16}H_{17}N_3O$
5j	$CH_2C_6H_{11}$	89	170–172 (C)	98		6.68		$C_{20}H_{21}N_3O$
5k	$CH_2C_6H_5$	86	180–182 (B)	97	86	5.61		$C_{20}H_{15}N_3O$
5l	$CH_2(3\text{-}ClC_6H_4)$	93	170–171 (B)	95		5.38		$C_{20}H_{14}ClN_3O$
5m	CH_2COCH_3	83	150–151 (C)	13		4.39		$C_{16}H_{13}N_3O_2$
5n	$(CH_2)_2OCH_3$	71	107–108 (C)	55		5.03		$C_{16}H_{15}N_3O_2$
5o	$(CH_2)_2OCOCH_3$	87	125–126 (C)	32		5.01		$C_{17}H_{15}N_3O_3$
5p	CH_2COOEt	85	180–181[h] (B)	28	0	4.36		$C_{17}H_{15}N_3O_3 \cdot {}^1/_6H_2O$
5q	$(CH_2)_3COOEt$	66	104–105 (C)	95		5.00		$C_{19}H_{19}N_3O_3$
5r	$CH_2C_6H_4$-4-COOMe	69	174–176 (B)	95		5.23		$C_{22}H_{17}N_3O_3$
5s	CO-2-thienyl	69	165–167 (B)	37		4.91		$C_{16}H_{11}N_3O_2S$
5t	$SO_2C_6H_5$	74	172–173 (C)	96		4.83		$C_{19}H_{13}N_3O_3S$
5u	$(CH_2)_2$-phthalimide	72	219–221 (B)	87		4.66		$C_{23}H_{16}N_4O_3$
7a	$(CH_2)_2$-1-tetrazole	41	141–142 (C)	43	77	4.62		$C_{16}H_{16}N_7O$[i]
7b	$(CH_2)_2SC_6H_5$	86	133–134 (C)		52	5.04		$C_{21}H_{17}N_3OS$
7c	$(CH_2)_2S$-2-thiazoline	51	123–124 (C)	97		4.98		$C_{16}H_{16}N_4OS_2$
7d	$(CH_2)_2S$-2-pyrimidine	79	145–146[h] (C)	86		4.96		$C_{19}H_{15}N_5OS$
7e	$(CH_2)_4S$-2-pyrimidine	92	117–118 (C)	96		5.21		$C_{21}H_{19}N_5O_3S$
8a	$(CH_2)_2OH$	92	172–174 (C)	69		5.34		$C_{15}H_{15}N_3O_2 \cdot {}^1/_6H_2O$
8b	CH_2COOH	81	241–242[h] (D)	13		<4.0		$C_{15}H_{11}N_3O_3 \cdot {}^2/_6H_2O$
8c	$(CH_2)_3COOH$	73	189–190 (D)	73		<4.0		$C_{17}H_{15}N_3O_3$
8d	$CH_2C_6H_4$-4-COOH	98	>300 (A)	64		4.77		$C_{21}H_{15}N_3O_3 \cdot {}^2/_3H_2O$
9	$(CH_2)_2NH_2$	87	162–163 (C)	29		<4.0		$C_{15}H_{14}N_4O$
10	$(CH_2)_2NHSO_2C_6H_5$	45	157–159 (C)	93		4.58		$C_{21}H_{18}N_4O_3S$
11	$(CH_2)_3CONHCH(CH_3)_2$	12	158–159 (C)	89		4.19		$C_{20}H_{22}N_4O_2$
aminophylline				0	0	4.33	86	
OKY-046				89	92	<3.0	0	

[a] Value of the final transformation was expressed. [b] Recrystallization solvent: A, EtOH–DMF; B, AcOEt–hexane; C, $CHCl_3$–hexane; D, EtOH. [c] At 1 h after administration. [d] Concentration activity curves were carried out with seven or more concentrations of test compounds, and IC_{50} values were calculated from log curve. [e] Inhibitory effects of test compounds on airway constriction induced by histamine 2–5 μg/kg iv at 1 min after 10 mg/kg iv administration of test compounds. [f] All compounds were analyzed for C, H, N, and results agreed to ±0.4% of theoretical values except for N of 7a. [g] See ref 1. [h] Decomposition. [i] N: calcd, 30.71; found, 30.15.

2-(methoxycarbonyl)pyrazine gave **14c**. The 5-pyrimidyl derivative (**14d**) was also prepared by reaction of the phthalic anhydride with the 5-pyrimidyllithium obtained from 5-bromopyrimidine and *n*-BuLi at −110 °C. Treatment of **14a–d** with hydrazine hydrate afforded the corresponding phthalazinones (**15a–d**), and certain alkyl substituents were introduced into the 2-position by the same procedure shown in Scheme I (Scheme VI).

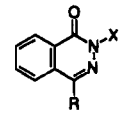
Pharmacological Results and Discussion

In order to test TXA_2 synthetase inhibitory activity, TXA_2 synthetase inhibition with enzyme from rabbit and rat serum TXA_2 production were employed. None of the test compounds inhibited PGI_2 formation as potently as those in the preceding paper.[1] Further, spontaneous tone inhibition with guinea pig tracheal strips and histamine-induced bronchoconstriction with anesthetized guinea pigs were employed to examine bronchodilatory activity.

Our hypothesis was that a 3-pyridyl moiety at the 4-position of compound 1 was able to generate TXA_2 synthetase inhibitory activity. Therefore, the 1-imidazolylalkyl moiety at the 2-position was not a necessary component in the 4-(3-pyridyl)phthalazinone system. Further, we expected that the removal of the imidazolyl group would lead to an increase of bronchodilatory activity by means of increased hydrophobicity, since we had found that the hydrophobicity of the compound significantly influenced bronchodilatory activity.[1] In order to explore these hypothesis, we evaluated both activities in the des(imidazolylethyl) (**4**) and desimidazolyl compounds (**5b**) (Table I). Compounds **4** and **5b** showed a higher bronchodilatory activity than the parent compound **1**, as expected. Further, compound **5b** was shown to have a TXA_2 synthetase inhibitory activity much higher than the parent compound **1**, beyond our expectations. The increased hydrophobicity due to the 2-ethyl group seemed to enhance the TXA_2 synthetase inhibition as well as bronchodilation. These findings prompted us to evaluate both activities in a number of 2-substituted 4-(3-pyridyl)-phthalazinones in an effort to find more potent compounds.

Among the examined substituents, the length and bulk of the alkyl groups have no influence on either activity,

Table II. 4-(3-Pyridyl)-1(2*H*)-phthalazinones



no.	R	X	yield[a] (%)	mp, °C (recrystn solvent)[b]	% inhibn of TXA_2 prodn *in vitro* at 1 μM	% inhibn of TXA_2 prodn *ex vivo*[c] 30 mg/kg po	bronchodilatory activity $-\log[IC_{50}(M)]$[d]	formula[e]
12a		H	34	287–289 (A)		87	5.08	$C_{11}H_8N_4O$
13a		C_2H_5	66	171–172 (B)	53	72	5.27	$C_{13}H_{12}N_4O$
12b		H	21	271–272 (A)			4.78	$C_{10}H_7N_5O$[f]
13b		C_2H_5	33	161–162 (B)	0		4.80	$C_{12}H_{11}N_5O$
15a		H	71	254–255 (C)		23	5.34	$C_{11}H_7N_3OS$
16a		C_2H_5	71	128–129 (B)	4	5	5.50	$C_{13}H_{11}N_3OS$
15b		H	78	229–230 (C)	38	70	5.92	$C_{11}H_7N_3OS$
16b		CH_3	74	198–200 (B)	55	83	5.80	$C_{12}H_9N_3OS$
16c		C_2H_5	83	121–122 (B)	77	69	6.25	$C_{16}H_{11}N_3OS$
16d		$CH(CH_3)_2$	57	74–75 (B)		71	5.47	$C_{14}H_{16}N_3OS$
16e		$CH_2CH(CH_3)_2$	89	85–86 (B)			5.62	$C_{15}H_{15}N_3OS$
16f		C_2H_5	68	146–147 (B)	37		4.96	$C_{14}H_{12}N_4O$
16g		C_2H_5	5[g]	202–204 (C)	26		5.02	$C_{14}H_{12}N_4O$

[a] Value of the final transformation was expressed except for **16g**. [b] Recrystallization solvent: A, EtOH; B, $CHCl_3$–hexane; C, EtOH–DMF. [c] See footnote c in Table I. [d] See footnote *d* in Table I. [e] All compounds were analyzed for C, H, N, and results agreed to ±0.4% of theoretical values except for N of **12b**. [f] N: calcd, 32.85; found, 32.40. [g] Total yield from 5-bromopyrimidine.

as shown in Table I. Although it had been reported that 1-alkylimidazoles exhibit an optimum effect on alkyl chain length,[5] it appeared that there is a bulk tolerance in the 2-substituents of 4-(3-pyridyl)phthalazinone. The ethyl group was found to be a sufficiently effective component for well-rounded and high potency. Introduction of certain functional groups, such as hydroxy, amino, carboxy, ester, sulfide, or sulfonyl groups, into the 2-alkyl substituents resulted in the reduction of bronchodilatory activity. As our previous findings had suggested that the introduction of hydrophilic substituents reduced the bronchodilatory activity, it was consequently hypothesized that the reduction of bronchodilatory activity resulted from the increase of hydrophilicity brought about by the introduction of the polar groups. Compounds having a heteroaromatic moiety (**5s**, **5u**, **7a**, and **7c–e**) showed less potent activities in both pharmacological tests than those having unsubstituted alkyl groups. From these results, we concluded that the most effective 2-substituent was an alkyl group, especially ethyl.

Subsequently, we evaluated both the TXA_2 synthetase inhibitory and bronchodilatory activities of 2-ethylphthalazinones having heteroaryl substituents other than a 3-pyridyl group at the 4-position and compared them with both activities of the 3-pyridyl derivative **5b**. Among these compounds, 1-imidazolyl (**13a**) and 5-thiazolyl (**16c**) derivatives were found to be as active as the parent compound **5b** for TXA_2 synthetase inhibition, but 1-triazolyl (**13b**), 2-thiazolyl (**16a**), 2-pyrazinyl (**16f**), and 5-pyrimidyl (**16g**) derivatives did not exhibit any significant activities. Taking into account reports that certain components, including the 1-imidazolyl,[5,6] 3-pyridyl,[2] and 5-thiazolyl[7] groups, are the basic structural requirements for giving a selective TXA_2 synthetase inhibition, the above observation strongly led us to consider the heteroaromatic nuclei at the 4-position of phthalazinone might play a critical role in TXA_2 synthetase inhibitory activity. On the other hand, in terms of bronchodilatory activity, the 5-thiazolyl derivative (**16c**) displayed the most potent activity, but the 1-imidazolyl derivative (**13a**) a lesser potency. These results are summarized in Table II, which also contains biological data on certain of the 2-unsubstituted and 2-methyl-, 2-isopropyl-, and 2-isobutyl-substituted compounds for reference. Since the log *P* values[8] of **5b**, **13a**, and **16c** were 1.76, 0.96, and 1.74, respectively, it appeared that the reduced bronchodilatory activity of **13a** is due to the increased hydrophilicity. The most effective 2-substituent of the 2-alkyl-4-(5-thiazolyl)-phthalazinones in terms of bronchodilation was the ethyl group, as had been observed for the 4-(3-pyridyl)-phthalazinones. However, preliminary studies on acute toxicity and others (data not shown) demonstrated that

Table III. TXA_2 Synthetase Inhibitory Activities of Highly Potent Compounds **5b** and **16b** and OKY-046

	IC_{50} (μM) *in vitro*[a]					% inhibn *ex vivo*[b]	
	human			rat			
compound	microsome	platelet	whole blood	platelet	whole blood	3 mg/kg	30 mg/kg
5b	1.6	27.9	2.3	1.3	1.7	66	92
16b	3.9	0.5	8.7	0.3	3.3	50	83
OKY-046	0.06	0.3	0.9	0.2	0.2	86	92

[a] IC_{50} values were determined from concentration activity curve which were carried out with five or more concentrations of test compounds and expressed as a mean of three or more different experiments. [b] Serum TXB_2 production at 1 h after oral administration of drug to rat (n = 6).

Table IV. Bronchodilatory Activities of Highly Potent Compounds **5b** and **16b** and Aminophylline

				in vivo			
	IC_{50} (μM) *in vitro*[a]			% inhibn		spasmogen inhalation induced collapse in conscious guinea pig: RD_{100} (mg/kg)[d]	
		spasmogen-contracted guinea pig trachea		histamine-induced bronchoconstriction in guinea pig at 10 mg/kg id[b]	anaphylactic bronchoconstriction in OA sensitized guinea pig at 10 mg/kg id[c]		
compound	spontaneous	ACh	histamine			ACh[e]	histamine[f]
5b	1.6	57	46	72	71	3.8	6.5
16b	1.8	46	28	68	61	6.6	11.2
aminophylline	44.0	300	320	87[g]	42	66.0	>60

[a] See footnote *d* in Table I. [b] Inhibitory effects of test compounds on airway constriction induced by histamine 2–5 μg/kg iv at 30 min after 10 mg/kg intraduodenum administration of test compounds. [c] Inhibitory effect of test compounds on airway constriction induced by antigen (OA) inhalation at 30 min after 10 mg/kg intraduodenum administration of test compounds. [d] Oral dose needed to prolong collapse time for 100 s from the vehicle response. [e] Acetylcholine (0.2%) was used and the mean time to collapse for control animals was 224 ± 20 s (mean ± SE, n = 36). [f] Histamine (0.03%) was used and the mean time to collapse for control animals was 207 ± 11 s (mean ± SE, n = 47). [g] Percent inhibition at a dose of 60 mg/kg.

16c had some undesirable properties. Among the other 4-(5-thiazolyl)phthalazinones, the 2-methyl derivative (**16b**) was also effective, although slightly less potent than **16c** in its bronchodilatory activity. Accordingly, we selected **5b** and **16b** for further pharmacological studies.

Pharmacological Activities of Compounds 5b and 16b

The TXA_2 synthetase inhibitory effects were measured using human and rat microsomes, intact platelets, and whole blood (Table III). OKY-046 inhibited human microsomes more effectively. On the other hand, compounds **5b** and **16b** manifested a different profile from that of OKY-046. Compound **5b** inhibited human platelets less effectively and compound **16b** inhibited more effectively in intact platelets of both the human and rat. The differences in the TXA_2 synthetase inhibitory profiles of these compounds suggest that they exhibit different pharmacodynamics of action.

The orally administered compounds **5b** and **16b** at a dose of 30 mg/kg inhibited TXA_2 production to almost the same degree as did OKY-046 in a rat *ex vivo* assay, although at a dose of 3 mg/kg compounds **5b** and **16b** inhibited less effectively than did OKY-046 (Table III).

The inhibitory effects of compounds **5b** and **16b** and aminophylline (the ethylenediamine salt of theophylline) on the contraction of guinea pig tracheal strips induced by several spasmogens were examined in addition to spontaneous contraction (Table IV). Aminophylline moderately inhibited contractions induced by acetylcholine or histamine. On the other hand, compounds **5b** and **16b** inhibited these contractions 5–10-fold more potently than did aminophylline at an almost equal concentration and, furthermore, inhibited spontaneous tone more effectively than aminophylline (20–30-fold more potent).

Intraduodenum administration of compounds **5b** and **16b** at a dose of 10 mg/kg prior to histamine challenge (Konzett–Rossler preparation) produced an inhibition of approximately 70%, although a dose of 60 mg/kg aminophylline was required to produce practically the same inhibitory activity (Table IV). The bronchoconstriction of sensitized guinea pigs induced by antigen inhalation was inhibited to 60–70% by intraduodenum administration of a 10 mg/kg dose of **5b** or **16b**. In histamine- and acetylcholine-induced bronchospasm tests using conscious guinea pigs, the orally administered compounds **5b** and **16b** were 10–20-fold more active than aminophylline (Table IV), and **5b** exhibited a bronchodilatory effect at a lower dose than did **16b**.

In conclusion, we found that 2-ethyl-4-(3-pyridyl)-1(2*H*)-phthalazinone (**5b**) (KK-505) and 2-methyl-4-(5-thiazolyl)-1(2*H*)-phthalazinone (**16b**) (KK-562) exhibited markedly high and well-rounded effects on both TXA_2 synthetase inhibition and bronchodilation. In general, agents possessing dual activities need to exhibit their dual activities at the same dose. Therefore, it is noteworthy that compounds **5b** and **16b** exhibited both TXA_2 synthetase inhibition and bronchodilation at almost the same dose in ranges of 3–30 mg/kg as described above. These compounds are now under development and are being investigated further for their pharmacological and toxicological effects.

Experimental Section

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 NMR spectra were taken with a Hitachi Model R-24B high-resolution magnetic resonance spectrometer (60 MHz) with tetramethylsilane as the 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 by a rotary evaporator.

Preparation of 2-Ethyl-4-(3-pyridyl)-1(2*H*)-phthalazinone (5b). 4-(3-Pyridyl)-1(2*H*)-phthalazinone (4) was prepared by the procedure described in a previous paper.[1] To a suspension of 60% NaH in oil (0.55 g, 13.2 mmol) in DMF (70 mL) was added **4** (2.5 g, 11.0 mmol), and the mixture was stirred for 30 min at room temperature under a nitrogen atmosphere. After

the mixture was cooled to 0 °C, ethyl bromide (1.25 mL, 16.5 mmol) was added, and the whole was stirred for 1 h at the same temperature. The mixture was poured into ice–water (100 mL) and extracted with $CHCl_3$. The extract was dried, concentrated, and chromatographed on silica gel with $CHCl_3$–acetone (99:1) to give 2.52 g (91%) of **5b** as white crystals. $CHCl_3$–hexane was used for recrystallization: mp 156–157 °C; IR (KBr) 1650 cm^{-1}; NMR ($CDCl_3$) δ 1.45 (3H, t, J = 7 Hz), 4.34 (2H, q, J = 7 Hz), 7.20–8.10 (5H, m), 8.31–8.90 (3H, m); MS m/e 251 (M^+, 93) 209 (100).

By the same procedure, **5a** and **5c–t** were prepared from **4**, and are summarized in Table I.

Preparation of 2-[2-(1-Tetrazolyl)ethyl]-4-(3-pyridyl)-1(2*H*)-phthalazinone (7a). To a suspension of 60% NaH in oil (0.4 g, 9.0 mmol) in DMF (50 mL) was added **4** (2.0 g, 6.7 mmol), and the whole was stirred for 30 min at room temperature under a nitrogen atmosphere. After the mixture was cooled to 0 °C, 1,2-dibromoethane (2.3 mL, 27 mmol) was added in one portion and stirred for 1 h at the same temperature. The mixture was poured into ice–water (200 mL) and extracted with $CHCl_3$. The extract was dried, concentrated, and chromatographed on silica gel with $CHCl_3$–acetone (49:1) to give 1.33 g (84%) of **6a** as white crystals. $CHCl_3$–hexane was used for recrystallization: mp 133–135 °C; IR (KBr) 1655 cm^{-1}; NMR ($CDCl_3$) δ 3.76 (2H, t, J = 7 Hz), 4.63 (2H, t, J = 7 Hz), 7.30–8.12 (5H, m), 8.34–8.92 (3H, m); MS m/e 331 (M^+, 30) 329 (M^+, 29) 223 (100). To a suspension of 60% NaH in oil (0.04 g, 1 mmol) in 10 mL of DMF was added 1-tetrazole (0.07 g, 1 mmol) and the mixture was stirred for 30 min at room temperature under a nitrogen atmosphere. **6a** (0.3 g, 0.9 mmol) was added, and the whole was stirred for 5 h at 50 °C. The mixture was poured into ice–water (200 mL) and extracted with $CHCl_3$. The extract was dried, concentrated, and chromatographed on silica gel with $CHCl_3$–MeOH (99:1) to give 0.12 g (41%) of **7a** as white crystals. $CHCl_3$–hexane was used for recrystallization: mp 141–142 °C dec; NMR ($CDCl_3$) δ 4.62–5.00 (2H, m), 5.11–5.43 (2H, m), 7.20–7.98 (5H, m), 8.40–8.92 (4H, m); MS m/e 319 (M^+, 18) 209 (100).

By the same procedure, compounds **7b–d** and **7e** were prepared from **6a** and **6b**, respectively, and are summarized in Table I. 2-(4-Bromobutyl)-4-(3-pyridyl)-1(2*H*)-phthalazinone (**6b**): yield 72%; mp 134–135 °C ($CHCl_3$–hexane); IR (KBr) 1645 cm^{-1}; NMR ($CDCl_3$) δ 1.60–2.38 (4H, m), 3.45 (2H, t, J = 7 Hz), 4.33 (2H, t, J = 7 Hz) 7.20–8.03 (5H, m), 8.32–9.00 (3H, m); MS m/e 359 (M^+, 4) 357 (M^+, 4) 278 (100).

Preparation of 2-(2-Hydroxyethyl)-4-(3-pyridyl)-1(2*H*)-phthalazinone (8a). A mixture of **5o** (0.35 g, 1.1 mmol) and 1 N NaOH (5 mL) in THF (10 mL) was stirred for 3 h at 40 °C. After cooling, the mixture was adjusted to pH 4 with 3 N HCl and extracted with THF. The extract was washed with brine, dried, concentrated, and chromatographed on silica gel with $CHCl_3$–MeOH (99:1) to give 0.27 g (92%) of **8a** as white crystals. $CHCl_3$–hexane was used for recrystallization: mp 172–174 °C; NMR ($CDCl_3$) δ 3.00 (1H, bs), 4.07 (2H, t, J = 5 Hz), 4.51 (2H, t, J = 5 Hz), 7.20–8.02 (5H, m), 8.40–8.98 (3H, m); MS m/e 268 (M^+ + 1, 7) 225 (100).

By the same procedure, compounds **8b–d** were prepared from **5p–r**, respectively, and are summarized in Table I.

Preparation of 2-(2-Aminoethyl)-4-(3-pyridyl)-1(2*H*)-phthalazinone (9). A mixture of **5u** (1 g, 2.5 mmol) and 80% hydrazine hydrate (0.47 g, 7.7 mmol) in EtOH (10 mL) was refluxed for 3 h under a nitrogen atmosphere. After the mixture was cooled to 0 °C, the resulting precipitates were filtered off, and the filtrate was concentrated. The residue was purified by chromatography on silica gel with $CHCl_3$–MeOH (19:1) to give 0.58 g (87%) of **9** as white crystals. $CHCl_3$–hexane was used for recrystallization: mp 162–163 °C; NMR ($CDCl_3$) δ 1.86 (2H, s), 3.00 (2H, t, J = 7 Hz), 4.20 (2H, t, J = 7 Hz), 7.40–8.18 (5H, m), 8.20–8.60 (1H, m), 8.62–8.90 (2H, m); MS m/e 266 (M^+, 0.2) 224 (100).

Preparation of 2-[2-[(Phenylsulfonyl)amino]ethyl]-4-(3-pyridyl)-1(2*H*)-phthalazinone (10). To a suspension of 60% NaH in oil (0.05 g, 1.3 mmol) in DMF (10 mL) was added **9** (0.3 g, 1.1 mmol) and the whole was stirred for 30 min at room temperature under a nitrogen atmosphere. After the mixture was cooled to 0 °C, benzenesulfonyl chloride (0.21 mL, 1.7 mmol) was added and the mixture stirred for 1 h at the same temperature. The mixture was poured into ice–water (100 mL) and extracted with $CHCl_3$. The extract was dried, concentrated, and chromatographed on silica gel with $CHCl_3$–MeOH (99:1) to give 0.2 g (45%) of **10** as white crystals. $CHCl_3$–hexane was used for recrystallization: mp 157–159 °C; IR 1650 cm^{-1}; NMR ($CDCl_3$) δ 3.51 (2H, t, J = 6 Hz), 3.60 (2H, t, J = 6 Hz), 5.88 (1H, t, J = 6 Hz), 7.10–8.12 (10H, m), 8.32–8.70 (1H, m), 8.70–9.03 (2H, m); MS m/e 406 (M^+, 4) 209 (100).

Preparation of 2-[3-[(*N*-Isopropylamino)carbonyl]propyl]-4-(3-pyridyl)-1(2*H*)-phthalazinone (11). To a solution of **8c** (0.29 g, 0.93 mmol) and triethylamine (0.14 mL, 1.0 mmol) in CH_2Cl_2 (20 mL) was added PCl_5 (0.21 g, 1.0 mmol), and the whole was stirred for 15 min at 0 °C. After the solvent was concentrated, DMF (10 mL) was added to the residue and cooled to 0 °C. Isopropylamine (0.18 mL, 2 mmol) was added to the mixture, and the whole was stirred for 1 h at room temperature. The mixture was poured into ice–water (50 mL) and extracted with $CHCl_3$. The extract was dried, concentrated, and chromatographed on silica gel with $CHCl_3$–MeOH (99:1) to give 0.04 g (12%) of **11** as white crystals. $CHCl_3$–hexane was used for recrystallization: mp 158–159 °C; IR 1665, 1635 cm^{-1}; NMR ($CDCl_3$) δ 1.13 (6H, d, J = 7 Hz), 2.14–2.35 (2H, m), 4.00 (1H, heptet, J = 7 Hz), 4.37 (2H, t, J = 7 Hz), 6.10 (1H, bs), 7.21–8.20 (5H, m), 8.38–9.11 (3H, m); MS m/e 350 (M^+, 26) 250 (100).

Preparation of 2-Ethyl-4-(1-imidazolyl)-1(2*H*)-phthalazinone (13a). To a suspension of 60% NaH in oil (0.55 g, 13.8 mmol) in dry DMF (100 mL) was added imidazole (0.78 g, 11.5 mmol), and the whole was stirred for 30 min at room temperature under a nitrogen atmosphere. After the mixture was cooled to 0 °C, 1,4-dichlorophthalazine (2.2 g, 11 mmol) was added and the mixture stirred for 2 h at room temperature, after which time 1 N NaOH (20 mL) was added to the mixture and the whole stirred for 1 h at the same temperature. After the mixture was concentrated, the residue was dissolved in a mixture of AcOEt (200 mL) and diluted HCl solution (200 mL), and the acidic aqueous layer was separated. The solution was adjusted to pH 8 with K_2CO_3 and left to stand at 4 °C overnight. The resulting precipitates were collected by filtration, washed with EtOH, and recrystallized from EtOH to give 0.8 g (34%) of **12a** as pale yellow crystals: mp 287–289 °C; IR (KBr) 1675 cm^{-1}; NMR (DMSO-d_6) δ 7.20–8.56 (7H, m), 11.95 (1H, bs); MS m/e 212 (M^+, 73) 103 (100). Introduction of an ethyl group into the 2-position of **12a** was performed as described above to give **13a** as white crystals. $CHCl_3$–hexane was used for recrystallization: yield 66%; mp 171–172 °C; IR (KBr) 1655 cm^{-1}; NMR ($CDCl_3$) δ 1.45 (3H, t, J = 7 Hz), 4.34 (2H, q, J = 7 Hz), 7.24–7.47 (2H, m), 7.53–8.12 (4H, m), 8.40–8.68 (1H, m); MS m/e 240 (M^+, 43) 130 (100).

By the same procedure, compounds **12b** and **13b** were prepared using triazole instead of imidazole and are summarized in Table II.

Preparation of 2-Methyl-4-(5-thiazolyl)-1(2*H*)-phthalazinone (16b). 2-(Trimethylsilyl)thiazole was prepared by following the literature procedure from thiazole: bp 66–68 °C (22 mmHg) (lit.[3] bp 58–60 °C (16 mmHg)). A solution of 2-(trimethylsilyl)-thiazole (19 g, 0.12 mol) in THF (150 mL) was added dropwise to a solution of 1.6 M *n*-butyllithium (in 76 mL hexane, 0.12 mol) at −78 °C under a nitrogen atmosphere and the whole stirred for 1.5 h at the same temperature. This reaction mixture was poured into a solution of phthalic anhydride (16.3 g, 0.11 mol) in THF (400 mL) in one portion at −78 °C under a nitrogen atmosphere, and the mixture was stirred for another hour at the same temperature. After the mixture was allowed to warm to room temperature, a mixture of 1% K_2CO_3(aq) and AcOEt was added, and the alkaline aqueous layer was separated. The aqueous layer was made acidic with 4 N HCl solution, and the whole was stirred for 30 min at room temperature. The solution was extracted with AcOEt (500 mL), and the extract was washed with water, dried, and evaporated. The residual solids were recrystallized from AcOEt to give 18.5 g (72%) of **14b** as white crystals: mp 146–148 °C; IR (KBr) 1695, 1670 cm^{-1}; NMR ($CDCl_3$–DMSO-d_6, 10:1) δ 7.43–8.26 (5H, m), 9.30 (1H, s), 10.75 (1H, bs); MS m/e 233 (M^+, 5) 57 (100). Treatment of **14b** as described above with hydrazine monohydrate gave **15b**: yield 78%; mp 229–230 °C (EtOH–DMF); IR (KBr) 1695 cm^{-1}; NMR (DMSO-d_6) δ 7.84–8.37 (4H, m), 8.41 (1H, s), 9.28 (1H, s), 13.05 (1H, bs); MS m/e 229 (M^+, 78) 145 (100). Treatment of **15b** with iodomethane in the presence of NaH afforded **16b**: yield 74%; mp 198–200 °C ($CHCl_3$–hexane); IR (KBr) 1660 cm^{-1}; NMR ($CDCl_3$) δ 3.90 (3H,

s), 7.76–8.15 (3H, m), 8.28 (1H, s), 8.49–8.68 (1H, m), 9.00 (1H, s); MS *m/e* 244 (M^+, 100).

Compounds **16c–e** were obtained by a similar method using the corresponding alkyl bromide and are summarized in Table II.

In a similar manner, compounds **14a–16a** were prepared using thiazole as the starting material instead of 2-(trimethylsilyl)-thiazole, and are summarized in Table II except for **14a**. 2-(2-Thiazonoyl)benzoic acid (**14a**): yield 43%; mp 172–173 °C (AcOEt); IR (KBr) 1705, 1675 cm^{-1}; NMR ($CDCl_3$–DMSO-d_6, 10:1) δ 7.37–8.06 (6H, m), 10.51 (1H, bs).

Preparation of 2-Ethyl-4-(2-pyrazinyl)-1(2*H*)-phthalazinone (16f). To a solution of 2-bromobenzoic acid (10.2 g, 51 mmol) in THF (300 mL) was added 1.6 M *n*-butyllithium (in 70 mL hexane, 110 mmol) dropwise at −78 °C under a nitrogen atmosphere, and the whole was stirred for 30 min at the same temperature. A solution of 2-(methoxycarbonyl)pyrazine [mp 59–60 °C (lit.[4] mp 60–60.5 °C)] (7.4 g, 53 mmol) was cooled to −50 °C and added to the reaction mixture in one portion at −78 °C under a nitrogen atmosphere, and the whole was stirred for 30 min at the same temperature. The mixture was allowed to warm to room temperature, quenched by the addition of water, and concentrated. The residual solids were dissolved with a mixture of AcOEt and 1% $NaHCO_3$, and the alkaline aqueous layer was separated. The alkaline aqueous layer was adjusted to pH 3 with diluted HCl and extracted with AcOEt. The extract was washed with brine, dried, concentrated, and chromatographed on silica gel with $CHCl_3$–MeOH (20:1) to give 2.5 g (23%) of **14c** as yellow solids: mp 191–194 °C; IR (KBr) 1690 cm^{-1}; NMR ($CDCl_3$–DMSO-d_6, 10:1) δ 7.42–7.85 (3H, m), 7.97–8.18 (1H, m), 8.40 (1H, bs), 8.51–8.78 (2H, m), 9.32 (1H, s). A solution of **14c** (2.5 g, 11 mmol) and 80% hydrazine hydrate (0.75 g, 12 mmol) in EtOH (100 mL) was refluxed for 3 h. The mixture was allowed to cool to room temperature and adjusted to pH 7 with diluted HCl. The resulting precipitates were collected by filtration, washed with EtOH, and recrystallized from EtOH to give 1.9 g (77%) of **15c** as white crystals: mp >300 °C; IR (KBr) 1690 cm^{-1}; NMR (DMSO-d_6) δ 7.85–8.10 (2H, m), 8.32–8.51 (2H, m), 8.78–8.90 (2H, m), 9.14 (1H, s), 13.10 (1H, bs). Anal. ($C_{12}H_8N_4O$) C, H, N. Treatment of **15c** with ethyl bromide in the presence of NaH gave **16f** as described above: yield 68%; mp 146–147 °C ($CHCl_3$–hexane); IR (KBr) 1650 cm^{-1}; NMR ($CDCl_3$) δ 1.48 (3H, t, J = 7 Hz), 4.42 (2H, q, J = 7 Hz), 7.70–7.97 (2H, m), 8.45–8.72 (2H, m), 8.71 (2H, s), 9.24 (1H, s).

Preparation of 2-Ethyl-4-(5-pyrimidyl)-1(2*H*)-phthalazinone (16g). To a solution of 5-bromopyrimidine (10 g, 50 mmol) in THF–Et_2O (1:1) (300 mL) was added a solution of 1.6 M *n*-butyllithium (in 35 mL hexane, 58 mmol) dropwise at −110 °C under a nitrogen atmosphere, and the whole was stirred for 30 min at the same temperature. A solution of phthalic anhydride (9 g, 60 mmol) in THF (400 mL) was cooled to −78 °C and poured into the reaction mixture in one portion at −78 °C under a nitrogen atmosphere, and the whole was stirred for 30 min at the same temperature. After the mixture was allowed to warm to room temperature, a mixture of 1% K_2CO_3 and AcOEt was added to the reaction mixture and the alkaline aqueous layer separated. The alkaline aqueous layer was made acidic with 4 N HCl solution, and the resulting precipitates were collected by filtration, washed with water, and dried to give 5.0 g of crude **14d** as yellow solids. A solution of crude **14d** (5 g, 22 mmol) and 80% hydrazine hydrate (1.6 g, 25 mmol) was refluxed for 3 h. The mixture was allowed to cool to 4 °C, and the resulting precipitates were collected by filtration, washed with EtOH, and dried to give 2.7 g (48%) of crude **15d** as white solids. These reactions were so complicated that we failed to purify **14d** as well as the phthalazinone (**15d**) derived from crude **14d**. Treatment of crude **15d** with ethyl bromide in the presence of NaH gave **16g** as described above: yield 5% from 5-bromopyrimidine; mp 202–204 °C ($CHCl_3$–hexane); IR (KBr) 1680 cm^{-1}; NMR ($CDCl_3$) δ 1.48 (3H, t, J = 7 Hz), 4.40 (2H, q, J = 7 Hz), 7.65–8.02 (3H, m), 8.49–8.68 (1H, m), 9.04 (2H, s), 9.37 (1H, s).

***In Vitro* Enzyme Assay of TXA_2 Synthetase.** Rabbit platelet microsomes as the enzyme source were prepared according to the methods of Needleman.[9] 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 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 subsequent reactions were carried out, as previously described. The percent inhibition of TXA_2 synthetase was calculated as relative to the content of TXB_2 in the control.

In vitro enzyme assay of human TXA_2 synthetase was carried out in a similar manner using commercially available human microsomes.

***In Vitro* Effects on TXB_2 Production Using Intact Platelets.** Anticoagulated human and rat blood with trisodium citrate was centrifuged to obtain platelet-rich plasma and resuspended in a Krebs-Henseleit buffer solution. Platelets (1×10^8 cell/mL) were used for TXB_2 production assay as described below.

***In Vitro* Effects on TXB_2 Production Using Whole Blood.** Non-anticoagulated human and rat blood was rapidly divided into 0.5-mL aliquots in glass tubes, each containing a single concentration of drug or corresponding vehicle equivalent to the highest concentration of each compound tested. Tubes were vortexed to ensure adequate mixing of the compound with the blood and then incubated in a water bath at 37 °C for 60 min to allow complete clot formation. Upon completion of the incubation period, blood samples were centrifuged to obtain 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.

***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 serum was deproteinized with EtOH, and the resulting supernatant was stored at −20 °C. The serum TXB_2 concentration was measured as described above.

Relaxation Effects 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 the 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, generally (apart from those 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.

In the experiments of agonist-induced tone, the tracheal strips were incubated with indomethacin (3 μM) for 60 min to inhibit spontaneous tone generated by prostaglandins, and the resting tension was set at 1 g. Inhibitory effects of the tested compounds on the contraction induced by acetylcholine (10^{-5} M final concentration) and histamine (10^{-4} M final concentration) were measured in the presence of indomethacin in a method similar to that described above.

Bronchoconstriction in Anesthetized Guinea Pigs. Male Dunkin–Hartley guinea pigs were anesthetized with ip 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 constantly with a pressure transducer. Histamine (1–5 μg/kg) was injected iv every 10 min through the jugular vein cannula to induce bronchoconstriction and administered repeatedly until a reproducible contraction (control response) was obtained. Tested compound (10 mg/kg) was administered iv 1 min before another challenge with histamine. The inhibitory effect of each compound was deter-

mined from three or more experiments and expressed as a mean percent inhibition of the control response.

In the experiment of intraduodenum administration, a catheter with a needle was inserted into the duodenum for id injection of drugs.

Anaphylactic Bronchoconstriction in Actively Sensitized Guinea Pigs. Male Hartley guinea pigs (300–400 g) were actively sensitized by ip and sc injection of 50 mg of ovalbumin (OA) 15 days before use. The animals were anesthetized with ip injected pentobarbital (35 mg/kg), and the insufflation pressure was measured by the method described above. Bronchoconstriction was induced with OA aerosols generated from an ultrasonic nebulizer (10 mg/mL, for 10 s) and were administered to the airways through a by-passed ventilatory system. Intraduodenum administration was carried out as described above.

Bronchoconstriction in Conscious Guinea Pigs. Male guinea pigs (400–500 g) were dosed orally with the compound or the vehicle and placed in individual chambers. At 1 h after drug administration, the animals were challenged for 9 min with a spasmogen aerosol generated from an ultrasonic nebulizer. The time from introduction of the aerosol to collapse was recorded, with those animals not collapsing within the 9-min observation time being considered as fully protected. The inhibitory effect of each compound was determined at three or more doses and expressed as a RD_{100} value which was the dose needed to prolong collapse time from the 100 s to vehicle response. In this experiment, collapsed animals were removed as soon as possible so the procedure would not prove to be fatal.

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