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

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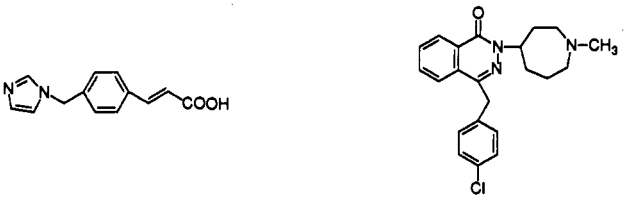
A number of 4-substituted 2-[ω-(1-imidazolyl)alkyl]-1(2*H*)-phthalazinones were synthesized in order to develop agents possessing both thromboxane A_2 synthetase inhibitory and bronchodilatory activities. The pharmacological evaluation of these compounds disclosed that they have both activities to various extents. Both activities were slightly dependent on the length of the 2-substituents and largely affected by the nature of the 4-substituents. Compounds bearing phenyl and thienyl groups exhibited relatively high and well-rounded activities. Among these compounds, **12j** and **15f** were found to be the most effective agents having well-rounded activities *in vitro* and *in vivo*. Introduction of a carboxyl group reduced both activities contrary to our expectation. 4-(3-Pyridyl)phthalazinone **18b** was of particular interest because of unexpectedly high *in vivo* activities in spite of an absence of significant *in vitro* activities.

Bronchial asthma is, in general, characterized by both bronchoconstriction and airway inflammation which leads to a bronchial hyperresponsiveness to various stimuli.[1] Bronchoconstriction is effectively inhibited by bronchodilators such as β_2-agonists and xanthine derivatives, and airway inflammation and bronchial hyperresponsiveness are well controlled by corticosteroids. However, bronchodilators are not effective for airway inflammation, and antiinflammatory drugs have no effect on bronchoconstriction.[2] At present, there are no available drugs effective for both bronchoconstriction and airway inflammation. Thus, combining the bronchodilatory and antiinflammatory activities in a single molecule has the potential to provide a drug markedly more effective in the treatment of bronchial asthma.

Although the mechanism of airway inflammation is not yet fully clarified, it has recently been found that thromboxane A_2 (TXA_2) plays an important role in the progressive development of airway inflammation and TXA_2 synthetase inhibitors may be useful in the treatment of asthma.[3] In particular, it was shown that OKY-046 (**1**, Figure 1),[4] a TXA_2 synthetase inhibitor, was effective in the clinical treatment of asthma and its effectiveness might be ascribed to its antiinflammatory action.[5] On the basis of such information, we focused concerted efforts on the development of a novel antiasthmatic agent with both TXA_2 synthetase inhibitory and bronchodilatory activities.

A number of compounds of diverse structure have already been reported to be potent TXA_2 synthetase inhibitors with the basic structural requirements being a 1-imidazolyl or a 3-pyridyl moiety at one end of the molecule and a carboxylic acid group at the other.[6] However, 1-nonylimidazole has been reported to be a very potent inhibitor in spite of the absence of a carboxylic acid group.[7] Further, it was recently reported that heterocyclic compounds having a 1-imidazolylalkyl moiety without a carboxylic acid group have TXA_2 synthetase inhibitory activity.[8] From these structural considerations, we selected the 1-imidazolylalkyl group as the necessary component for TXA_2 synthetase inhibition. We also noticed that azelastine (**2**)[9] might serve as a useful parent compound possessing bronchodilatory activity. This compound is an antiallergic phthalazinone derivative reported to have bronchodilatory activity in a clinical study.[10] Thus, we selected the phthalazinone skeleton as the fundamental structure effective for bronchodilation.



1 OKY-046 **2** azelastine

Figure 1.

This paper describes the synthesis and pharmacological evaluation of phthalazinone derivatives having imidazolylalkyl groups as compounds with both TXA_2 synthetase inhibitory and bronchodilatory activities.

Chemistry

Introduction of ω-(1-imidazolyl)alkyl groups into the 2-position of the 1(2*H*)-phthalazinone skeleton was carried out by two different methods, as illustrated by 4-phenyl-1(2*H*)-phthalazinone (**4**) in Scheme I. One is a one-pot process involving successive treatment with α,ω-dibromoalkanes and imidazole in the presence of potassium carbonate (method I), and the other a direct alkylation by reaction with 1-(2-bromoethyl)imidazole[11] in the instances of the introduction of the 2-(1-imidazolyl)ethyl group (method II).

In order to introduce a variety of substituents into the 4-position of 1(2*H*)-phthalazinone, we employed various 2-acylbenzoic acids (**7a–d**, **10a–l**, **13a–h**, **16a–c**) as precursors of 4-substituted 1(2*H*)-phthalazinones. Besides the commercially available 2-acylbenzoic acids (**7a**, **10a–d**), desired 2-acylbenzoic acids were prepared from phthalic anhydride by the following three methods: Friedel–Crafts acylation (**10e**, **10h–l**, **13a**, **13c–g**) (method A), Grignard reaction (**7b–d**, **10f–g**) (method B), and reaction with lithio compounds (**13b**, **13h**, **16a–c**) (method C). These 2-acylbenzoic acids readily underwent cyclization upon

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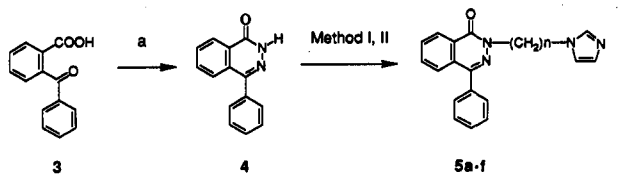
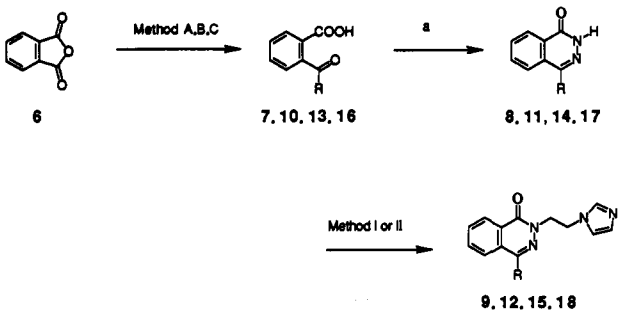
Scheme I[a]



5a	n=2	Method I and II
b	n=3	Method I
c	n=4	Method I
d	n=5	Method I
e	n=6	Method I
f	n=8	Method I

[a] (a) H_2NNH_2, EtOH, reflux, 92% yield; method I, (i) $Br(CH_2)_n$-Br, K_2CO_3, DMF, 60 °C; (ii) imidazole; method II, (bromoethyl)imidazole, K_2CO_3, DMF, 80 °C.

Scheme II[a]



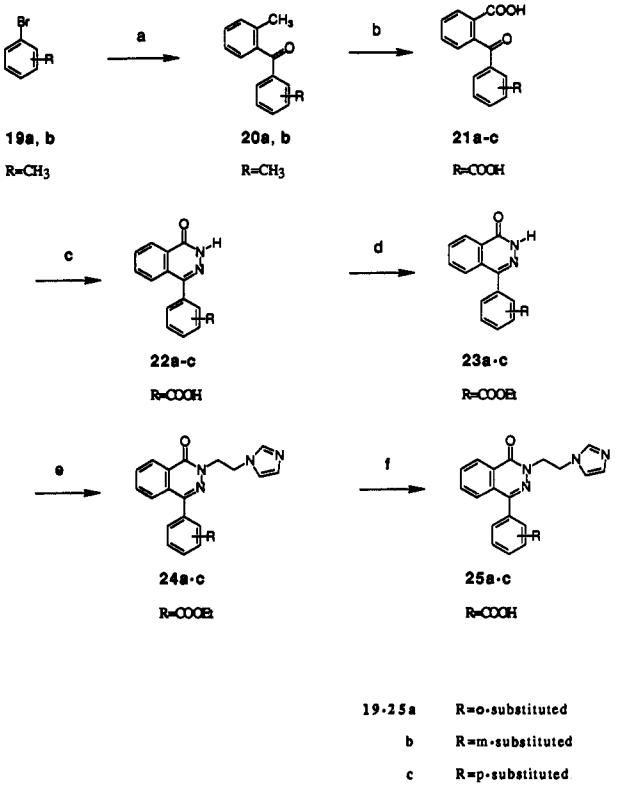
7-9a	R=Me	13-15a	R=2-thienyl (Method A)
b	R=isobutyl (Method B)	b	R=2-furyl (Method C)
c	R=cyclopentyl (Method B)	c	R=2-(5Cl-thienyl) (Method A)
d	R=cyclohexyl (Method B)	d	R=2-(5Me-thienyl) (Method A)
		e	R=2-(3Et-thienyl) (Method A)
10-12a	R=4F-Ph	f	R=2-(5Et-thienyl) (Method A)
b	R=4Cl-Ph	g	R=2-(5MeO-thienyl) (Method A)
c	R=4Me-Ph	h	R=2-(5Ac-thienyl) (Method C)
d	R=4Et-Ph		
e	R=4isoPr-Ph (Method A)	16-18a	R=2-pyridyl (Method C)
f	R=3CF3-Ph (Method B)	b	R=3-pyridyl (Method C)
g	R=4CF3-Ph (Method B)	c	R=4-pyridyl (Method C)
h	R=4MeO-Ph (Method A)		
i	R=4EtO-Ph (Method A)		
j	R=MeS-Ph (Method A)		
k	R=3,4(MeO)2-Ph (Method A)		
l	R=3F,4MeO-Ph (Method A)		

[a] Method A, RH, $AlCl_3$; method B, R-Mg-halogen; method C, RH or Br, *n*-BuLi, −78 °C, THF; (a) H_2NNH_2, EtOH, reflux; method I, (i) $Br(CH_2)_nBr$, K_2CO_3, DMF, 60 °C; (ii) imidazole; method II, (bromoethyl)imidazole, K_2CO_3, DMF, 80 °C.

treatment with hydrazine hydrate in ethanol to give the corresponding 4-substituted phthalazinones (**8a–d**, **11a–1**, **14a–h**, **17a–c**), which afforded the desired 2-substituted phthalazinones (**9a–d**, **12a–l**, **15a–h**, **18a–c**) by method I or II (Scheme II).

4-[(Ethoxycarbonyl)phenyl]- and 4-(carboxyphenyl)-1(2*H*)-phthalazinones (**24a–c** and **25a–c**) were similarly prepared from the corresponding (carboxybenzoyl)benzoic acids (**21a–c**). Compounds **21a** and **21b** were obtained by *n*-butyllithium-mediated reactions of *o*-tolunitrile with 2- and 3-bromotoluenes, followed by oxidation with $KMnO_4$.

Scheme III[a]



19-25a	R=o-substituted
b	R=m-substituted
c	R=p-substituted

[a] (a) (i) *n*-BiLi, −78 °C, THF; (ii) *o*-tolunitrile; (iii) H^+; (b) $KMnO_4$, pyridine–H_2O, reflux; (c) H_2NNH_2, EtOH, reflux; (d) EtOH, H^+; (e) (bromoethyl)imidazole, K_2CO_3, DMF, 80 °C; (f) NaOH (aq).

Compound **21c** was commercially available (Scheme III). Transformation of **21a–c** into **24a–c** and **25a–c** was effected by the following processes: (1) cyclization with hydrazine hydrate to the phthalazinones (**22a–c**); (2) esterification with EtOH–H_2SO_4 to the ethyl esters (**23a–c**); (3) 2-(1-imidazolyl)ethylation by method II to **24a–c**; (4) alkaline hydrolysis of **24a–c** to **25a–c** (Scheme III).

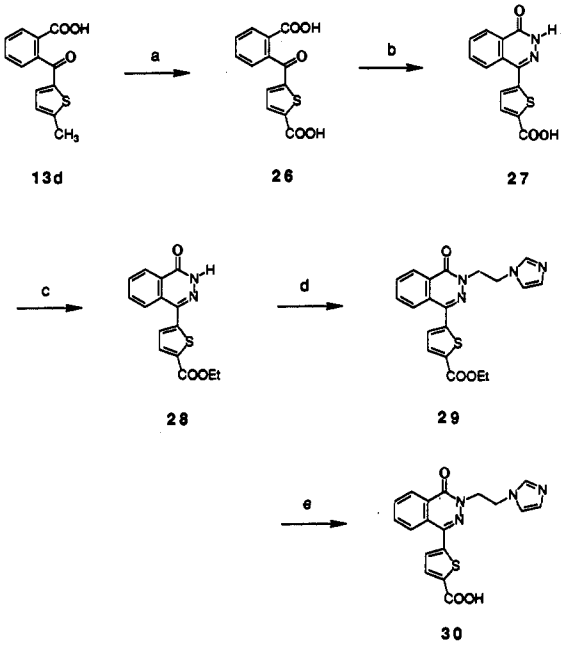
5-(Ethoxycarbonyl)- and 5-carboxy-2-thienyl derivatives (**29** and **30**) were also prepared in a similar way from the carboxythienoylbenzoic acid (**26**) obtainable by $KMnO_4$ oxidation of **13d** (Scheme IV).

Further, 4-benzyl- and 4-(2-thienylmethyl)phthalazinone derivatives (**33a–c**) were synthesized from phthalic anhydride according to the known procedure[12] with slight modification. Treatment of phthalic anhydride with substituted acetic acids at high temperature (180–200 °C) gave rise to condensation products (**31a–c**) accompanied by decarboxylation. Cyclization of **31a–c** with hydrazine hydrate followed by imidazolylethylation by method II afforded **33a–c**.

Pharmacological Results and Discussion

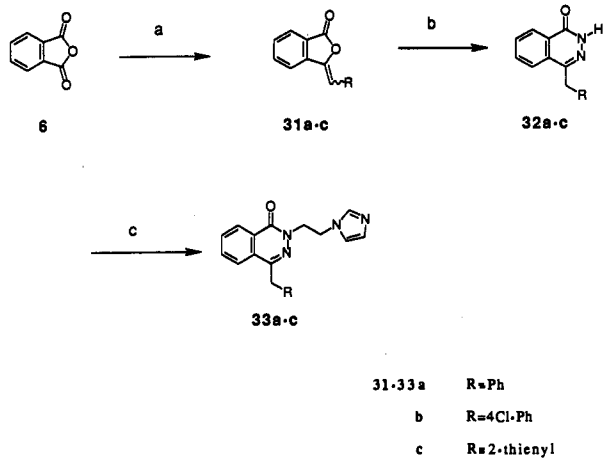
The compounds prepared in this study were tested for TXA_2 synthetase inhibition with enzyme from rabbit as *in vitro* assay and rat serum TXA_2 production as *ex vivo* assay. Also, as in earlier reports,[8] all of the compounds were evaluated for their selectivity by examining their effects on PGI_2 formation in pig aortic rings. None of the test compounds inhibited PGI_2 formation. This result is consistent with a mechanism of selective TXA_2 synthetase inhibition. For bronchodilatory activity, we tested spontaneous tone inhibition with guinea pig tracheal strips as an *in vitro* assay and inhibitory effect on histamine-induced

Scheme IV[a]



[a] (a) $KMnO_4$, pyridine–H_2O, reflux, 52% yield; (b) (i) H_2NNH_2, EtOH, reflux, 65% yield; (c) EtOH, H^+, 59% yield; (d) (bromoethyl)imidazole, K_2CO_3, DMF, 80 °C, 73% yield; (e) NaOH (aq), 83% yield.

Scheme V[a]

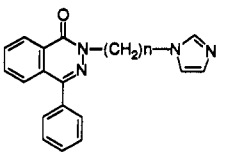


[a] (a) RCH_2COOH, CH_3COONa, DMA, reflux; (b) H_2NNH_2, EtOH, reflux; (c) (bromoethyl)imidazole, K_2CO_3, DMF, 80 °C.

bronchoconstriction using anesthetized guinea pigs as an *in vivo* assay.

In order to examine the possibility of developing compounds possessing both TXA_2 synthetase inhibitory and bronchodilatory activities by the introduction of ω-(1-imidazolyl)alkyl groups into the phthalazinone skeleton, we chose 4-phenyl-1(2*H*)-phthalazinone as the basic compound and synthesized several 2-substituted derivatives with ω-(1-imidazolyl)alkyl groups of different chain lengths (**5a–f**) (Table I). These compounds showed both TXA_2 synthetase inhibitory and bronchodilatory activities *in vitro* as expected. TXA_2 synthetase inhibitory activity was dependent on the length of the alkyl chain, and maximum activity was obtained with a compound of n = 6 (**5e**). The observation that the inhibitory potency of such compounds reaches an optimum value is consistent with descriptions of other compound systems in the literature.[5-8] In terms of bronchodilatory activity, maximum response was obtained with a compound of n = 2

Table I. 2-[2-(1-Imidazolyl)alkyl]-4-phenyl-1(2*H*)-phthalazinones



no.	n	yield[a] (%)	mp, °C (recrystn solvent)[b]	% inhbn of TXA_2 prodn at 1 μM	broncho-dilatory activity $-\log[IC_{50}\ (M)]$[c]	formula[d]
5a	2	28	164–165 (A)	40	5.58	$C_{19}H_{16}N_4O$
5b	3	22	136–138 (A)	57	4.96	$C_{20}H_{16}N_4O$
5c	4	32	145–146 (A)	65	4.76	$C_{21}H_{20}N_4O$
5d	5	26	164–165 (B)	69	4.63	$C_{22}H_{22}N_4O$
5e	6	24	101–103 (B)	79	5.01	$C_{23}H_{24}N_4O$
5f	8	21	81–82 (B)	63	5.09	$C_{25}H_{28}N_4O$

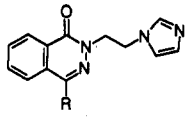
[a] Prepared by method I. [b] Recrystallization solvent: A, AcOEt–hexane; B, $CHCl_3$–hexane. [c] Concentration activity curves were carried out with seven concentrations of test compounds, and IC_{50} values were calculated from log curve. [d] All compounds were analyzed for C, H, and N, and results agreed to ±0.4% of theoretical values.

(**5a**). Although the most effective length of the alkyl chain was different for the TXA_2 synthetase inhibitory and bronchodilatory activities, these data indicate that introduction of ω-(1-imidazolyl)alkyl groups into the 2-position of the phthalazinone skeleton is able to yield both activities. From these findings, we selected the 2-(1-imidazolyl)ethyl moiety as the common 2-substituent and tried to increase both activities by modification of other regions of the molecule.

We examined the relationship between the structure of the 4-substituents and the respective activities (Table II). While compounds having short alkyl groups at the 4-position (**9a** and **9b**) did not demonstrate any significant activity in either TXA_2 synthetase inhibition or bronchodilation, those with cycloalkyl groups, such as **9c** and **9d**, were found to have high bronchodilatory and moderate TXA_2 synthetase inhibitory activities. Compounds **5a** and **12b**, which have a directly-bound phenyl group, exhibited a higher potency in both activities *in vitro* than the corresponding methylene inserted compounds (**33a** and **33b**). Compound **12b** was, in particular, shown to have a higher potency than the parent compound **33b**, which more closely resembled azelastine in structure. Methylene-inserted compounds such as **33a–c** showed a high bronchodilatory activity *in vivo*, but were relatively less active *in vitro* (Tables II and IV). In further studies, we found that these compounds possessed antihistaminergic activity. As we employed an assay system in which the inhibitory effect of compounds on bronchoconstriction induced by histamine iv administration was measured as a test of *in vivo* bronchodilatory activity, it was suggested that the *in vivo* effect of methylene-inserted compounds resulted from the antihistaminergic rather than bronchodilatory activity. These findings suggest that a directly bound aromatic ring was required for desirable and well-rounded activities. On the basis of these observations, we focused on the synthesis of phenyl-, thienyl-, furyl-, and pyridylphthalazinone derivatives and further introduction of certain functional groups into the 4-aromatic substituents.

Among the 4-phenylphthlazinone derivatives, the 4-(methylthio)phenyl derivative **12j** exhibited the highest activities in both the *in vitro* and *in vivo* pharmacological tests (Table III). Introduction of a carboxylic acid (**25a–c**) surprisingly resulted in the reduction of not only the TXA_2 synthetase inhibitory activity but the broncho-

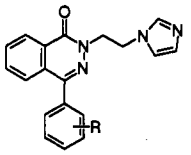
Table II. 2-[2-(1-Imidazolyl)ethyl]-1(2*H*)-phthalazinones



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]
9a	methyl	72	89–90 (A)	10		<4.0		$C_{14}H_{14}N_4O$
9b	isobutyl	44	97–98 (B)	14	21	4.92		$C_{17}H_{20}N_4O$
9c	cyclopentyl	48	102–103 (B)	38		5.23	98	$C_{18}H_{20}N_4O$
9d	cyclohexyl	45	151–153 (B)	32	28	6.17	71	$C_{19}H_{22}N_4O \cdot {}^1/_3H_2O$
5a	phenyl	82	164–165 (A)	44	0	5.88	88	$C_{18}H_{18}N_4O$
12b	4-Cl-phenyl	82	199–200 (A)	63	40	5.69	71	$C_{18}H_{15}ClN_4O$
33a	benzyl	67	127–128 (B)	45	−5	4.97	98	$C_{20}H_{18}N_4O$
33b	4-Cl-benzyl	72	150–151 (B)	49	40	5.25	100	$C_{20}H_{17}ClN_4O$
aminophylline				0	0	4.33	86	
OKY-046				89	92	<3.0	0	

[a] Prepared by method II. [b] See footnote *b* in Table I. [c] At 1 h after administration. [d] See footnote *c* in Table I. [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] See footnote *d* in Table I.

Table III. 2-[2-(1-Imidazolyl)ethyl]-1(2*H*)-phthalazinones



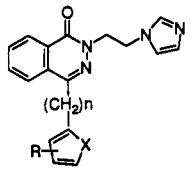
no.	R	yield[a] (%)	mp, °C (recrystn solvent)[b]	% inhibn of TXA_2 prodn: *in vitro* at 1 μM	% inhibn of TXA_2 prodn: *ex vivo*[c] 30 mg/kg po	bronchodilatory activity: *in vitro*[d] −log[IC_{50} (M)]	bronchodilatory activity: *in vivo*[e] % inhibn	formula[f]
5a	H	83	164–165 (A)	41	0	5.88	88	$C_{18}H_{18}N_4O$
12a	4-F	75	195–197 (A)	33	46	4.80	100	$C_{18}H_{15}FN_4O$
12b	4-Cl	82	199–200 (A)	63	40	5.69	71	$C_{18}H_{15}ClN_4O$
12c	4-Me	74	177–178 (B)	76	0	5.29	94	$C_{20}H_{18}N_4O$
12d	4-Et	70	133–135 (B)	79	55	5.11	8	$C_{21}H_{20}N_4O$
12e	4-i-Pr	67	132–134 (A)	64	52	5.27	68	$C_{22}H_{22}ClN_4O$
12f	3-CF_3	42	126–128 (A)	49	37	6.00	42	$C_{20}H_{15}F_3N_4O$
12g	4-CF_3	56	172–174 (A)	40		4.95		$C_{20}H_{15}F_3N_4O$
12h	4-OMe	72	150–151 (B)	74	50	4.60	0	$C_{20}H_{18}N_4O_2$
12i	4-OEt	68	137–139 (B)	54	68	5.25	87	$C_{21}H_{20}N_4O_2$
12j	4-SMe	71	168–169 (B)	83	67	5.35	73	$C_{20}H_{18}N_4OS$
12k	3,4-$(OMe)_2$	36	134–135 (B)	27	32	5.19	90	$C_{21}H_{20}N_4O_3$
12l	3-F-4-OMe	24	128–130 (A)	63	60	5.41	54	$C_{20}H_{17}FN_4O_2$
24a	2-COOEt	76	127–128 (B)	64		4.22	60	$C_{22}H_{20}N_4O_3$
25a	2-COOH	78	>300 (solids)	14		4.31	94	$C_{20}H_{18}N_4O_3$
24b	3-COOEt	78	116–117 (B)	55	0	5.63	90	$C_{22}H_{20}N_4O_3$
25b	3-COOH	71	275–277 (solids)	10		<4.0	33	$C_{20}H_{18}N_4O_3 \cdot H_2O$
24c	4-COOEt	64	161–162 (B)	60		5.00	92	$C_{22}H_{20}N_4O_3$
25c	4-COOH	78	>300 (solids)	24		4.19	21	$C_{20}H_{18}N_4O_3$

[a] Prepared by method II except for **12k** and **12l** (method I). [b] See footnote *b* in Table I. [c] See footnote *c* in Table II. [d] See footnote *c* in Table I. [e] See footnote *e* in Table II. [f] See footnote *d* in Table I.

dilatory activity as well, although a carboxylic acid group, in general, would be expected to enhance TXA_2 synthetase inhibitory activity.[6] The ester derivatives (**24a–c**) exhibited a relatively higher potency than the corresponding carboxylic acids derivatives (**25a–c**) in both pharmacological tests.

We also examined the activities of compounds having a thienyl group, a bioisostere of a phenyl group, and compounds having a furyl group as a structurally related heteroaromatic ring (Table IV). Compound **15a**, having the 2-thienyl group, exhibited a moderate potency in both pharmacological tests, while the 2-furyl derivative **15b** was less active in the bronchodilatory activity test. Among 4-(2-thienyl)phthalazinone derivatives having certain substituents on the thiophene ring, the 5-ethylthienyl derivative **15f** was shown to possess the highest activity in both assays. In the same manner as the phenyl derivatives, introduction of a carboxylic acid into the thiophene ring (**30**) led to the loss of bronchodilatory activity, and esterification of the carboxylic acid (**29**) resulted in both activities being retained to some degree. The reason for the absence of any enhancement of the TXA_2 synthetase inhibitory activity by the introduction of a carboxylic acid group could not be clarified in this limited study. Taking into account that 1-nonylimidazole[7] or other compounds[8] without carboxylic acid groups have been reported to be very potent inhibitors, it was believed that the hydrophobicity of the compounds might also play an important role in the TXA_2 synthetase inhibitory activity in this phthalazinone system. Further, it is noteworthy that the introduction of a carboxylic group led to the reduction of bronchodilatory activity. These findings suggest that the bronchodilatory activity is also influenced by hydrophobicity of the compound. In fact, comparison of the

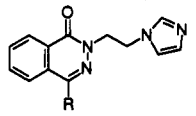
Table IV. 2-[2-(1-Imidazolyl)ethyl]-1(2*H*)-phthalazinones



no.	R	X	n	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]
15a	H	S	0	82	132–134 (A)	22	47	5.45	84	$C_{17}H_{14}N_4OS$[g]
15b	H	O	0	56	142–143 (A)	27		4.37		$C_{17}H_{14}N_4O_2$
15c	5-Cl	S	0	74	138–140 (A)	47	15	5.84	96	$C_{17}H_{13}ClN_4OS$
15d	5-Me	S	0	67	130–131 (A)	53	0	5.18	26	$C_{18}H_{16}N_4OS$
15e	3-Et	S	0	53	130–131 (A)	68	46	4.73	84	$C_{18}H_{16}N_4OS \cdot {}^2/_3H_2O$
15f	5-Et	S	0	73	110–112 (A)	67	52	5.75	96	$C_{19}H_{18}N_4OS$
15g	5-OMe	S	0	64	113–114 (A)	88		5.37		$C_{18}H_{16}N_4O_2S \cdot {}^1/_2H_2O$
15h	5-Ac	S	0	67	139–140 (A)	80	5	4.82	94	$C_{19}H_{16}N_4O_2S$[h]
29	5-COOEt	S	0	73	130–131 (B)	62		4.88	92	$C_{20}H_{16}N_4O_3S$
30	4-COOH	S	0	83	242–244 (solids)	38		4.46	5	$C_{18}H_{14}N_4O_3S$
33c	H	S	1	42	135–137 (B)	29	35	5.12	100	$C_{18}H_{16}N_4OS$

[a] See footnote *a* in Table II. [b] See footnote *b* in Table I. [c] See footnote *c* in Table II. [d] See footnote *c* in Table I. [e] See footnote *e* in Table II. [f] All compounds were analyzed for C, H, N, and results agreed to ±0.4% of theoretical values except for N of **15a** and C of **15h**. [g] N: calcd, 17.38; found, 16.87. [h] C: calcd, 62.62; found, 62.11.

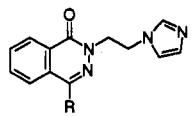
Table V. 2-[2-(1-Imidazolyl)ethyl]-1(2*H*)-phthalazinones



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]
18a	2-pyridyl	74	113–115 (B)	20	41	4.37	30	$C_{18}H_{15}N_5O$
18b	3-pyridyl	57	147–148 (A)	38	90 (63[g])	4.57	97	$C_{18}H_{15}N_5O$
18c	4-pyridyl	57	170–171 (A)	27	47	4.62	100	$C_{18}H_{15}N_5O$

[a] See footnote *a* in Table II. [b] See footnote *b* in Table I. [c] See footnote *c* in Table II. [d] See footnote *c* in Table I. [e] See footnote *e* in Table II. [f] See footnote *d* in Table I. [g] Percent inhibition at an oral dose of 3 mg/kg.

Table VI. Relationship between Bronchodilatory Activity and log *P* Value



no.	R	bronchodilatory activity −log[IC_{50} (M)][a]	log *P*[b]	no.	R	bronchodilatory activity −log[IC_{50} (M)][a]	log *P*[b]
9a	methyl	<4.0	0.538	**12d**	4-Et-phenyl	5.11	3.165
9b	isobutyl	4.92	1.995	**12h**	4-MeO-phenyl	4.60	2.056
9c	cyclopentyl	5.23	2.629	**12j**	4-MeS-phenyl	5.35	2.696
9d	cyclohexyl	6.17	3.188				
				15a	2-thienyl	5.45	1.993
5a	phenyl	5.88	2.137	**15b**	2-furyl	4.37	1.523
18a	2-pyridyl	4.37	0.870				
18b	3-pyridyl	4.57	0.660	**15f**	2-(5-Et-thienyl)	5.75	3.021
18c	4-pyridyl	4.62	0.660	**15g**	2-(5-MeO-thienyl)	5.37	1.937
				15h	2-(5-Ac-thienyl)	4.82	1.508

[a] See footnote c in Table I. [b] Calculated using MedChem System.[13]

bronchodilatory activities of structurally related derivatives showed that a decrease of log *P* values[13] of these compounds brought about a reduction of bronchodilatory activities, as shown in Table VI. These data support the idea that the hydrophobicity of these compounds has an important influence on not only the TXA_2 synthetase inhibition but also bronchodilation in this phthalazinone system.

In the case of the 4-pyridylphthalazinones, none of the tested compounds (**18a–c**) exhibited any significant activities, probably due to the decrease of their hydrophobicity in these instances as well. However, very interestingly, 4-(3-pyridyl)phthalazinone **18b** showed a considerable potency in both the *in vivo* tests in spite of having low potency *in vitro* (Table V). In particular, **18b** exhibited the most effective TXA_2 synthetase inhibitory activity *in vivo* among any of the compounds tested in this study. The TXA_2 synthetase inhibitory activity of **18b** at a dose of 3 mg/kg is equivalent to that of OKY-046 at a dose of 1 mg/kg. It is evident that **18b** is still less active than newly developed specific TXA_2 synthetase inhibitors such as CV-4151,[6,14] however, the bronchodilatory activity of

18b is more potent than that of aminophylline. Thus, 18b has been shown to have the most desirable and well-rounded pharmacological effects among the tested compounds in this study, at least *in vivo*.

The mechanism of bronchodilation in these compounds remains to be elucidated. However, taking into account the chemical structures, these compounds could be thought to comprise a new category different from β-stimulants or xanthine derivatives. Although the activities of these compounds are not remarkably potent, especially in terms of TXA_2 synthetase inhibitory activity, it is noteworthy that novel compounds with well-rounded activities such as 18b are being developed as prototypes for new compounds with more potent dual activities.

In conclusion, 4-substituted 2-[ω-(1-imidazolyl)ethyl]-1(2*H*)-phthalazinones have been shown to be generally effective for TXA_2 synthetase inhibition and bronchodilation. Certain compounds in this study, such as 12j, 15f, and 18b, have been found to be potent agents with well-rounded dual activities. It has been further shown that, in this phthalazinone system, the hydrophobicity of the compounds has a marked influence on both activities. Of particularly interest is 4-(3-pyridyl)phthalazinone (18b), which exhibits an unexpectedly high degree of both activities *in vivo* in spite of lacking significant activities *in vitro*. Further studies on the pharmacological effects of 18b and related compounds will be reported in a subsequent paper.

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-[2-(1-Imidazolyl)ethyl]-4-phenyl-1(2*H*)-phthalazinone (5a) (Method I). A solution of 2-benzoylbenzoic acid (3) (5.0 g, 22.1 mmol) and 80% hydrazine hydrate (1.8 g, 29 mmol) in EtOH (200 mL) was refluxed for 5 h. After cooling, the resulting precipitates were filtered, washed with EtOH, and recrystallized from EtOH–DMF to give 4.5 g (92%) of 4-phenyl-1(2*H*)-phthalazinone (4) as white crystals: mp 233–235 °C. To a suspension of 4 (2.0 g, 9.0 mmol) and K_2CO_3 (7.5 g, 54 mmol) in dry DMF (60 mL) was added 1,2-dibromoethane (5.5 g, 29 mmol), and the mixture was stirred for 1 h at 60 °C. Imidazole (4.3 g, 63 mmol) was added, and the whole was stirred for 3 h at the same temperature. To the cooled mixture were added 2 N HCl and AcOEt, and the acidic aqueous layer was separated, made alkaline with 5% K_2CO_3 solution, and extracted with AcOEt (300 mL). The extract was washed with brine, dried, and concentrated under reduced pressure. The residue was purified by chromatography on silica gel with $CHCl_3$–MeOH (20:1) and recrystallized from AcOEt–hexane to give 0.8 g (28%) of 5a as white crystals: mp 164–165 °C; IR (KBr) 1660 cm^{-1}; NMR ($CDCl_3$) δ 4.40–4.71 (4H, m), 6.81–7.92 (11H, m), 8.29–8.61 (1H, m).

By the same procedure, 5b–f were prepared from 4 using α,ω-dibromoalkanes, and these are summarized in Table I.

Preparation of 2-[2-(1-Imidazolyl)ethyl]-4-phenyl-1(2*H*)-phthalazinone (5a) (Method II). 1-(2-Bromoethyl)imidazole hydrogen bromide was prepared according to the known procedure[11] with slight modification. Thus, a mixture of imidazole (26 g, 0.38 mol) and ethylene carbonate (52 g, 0.59 mol) in toluene (100 mL) was refluxed for 5 h. The mixture was cooled to room temperature, and the resulting upper layer was removed by decantation. To the residual layer was added concentrated HCl (100 mL), and the mixture was washed with $CHCl_3$. The aqueous layer was made alkaline with K_2CO_3 and extracted with $CHCl_3$ (200 mL, three times). The extract was dried, concentrated, and chromatographed on silica gel with $CHCl_3$–MeOH (20:1) to give 18.3 g (43%) of 1-(2-hydroxyethyl)imidazole as a colorless oil: NMR ($CDCl_3$) δ 3.68–4.12 (4H, m), 5.71 (1H, bs), 6.81 (1H, s), 6.86 (1H, s), 7.37 (1H, s). To a solution of 1-(2-hydroxyethyl)-imidazole (12 g, 0.11 mol) in CH_2Cl_2 (200 mL) was added dropwise at 15 °C a solution of thionyl bromide (52 g, 0.12 mol) in CH_2Cl_2 (10 mL), and the whole was stirred for 1 h at room temperature. The mixture was concentrated under reduced pressure, and the residue was dissolved in EtOH (50 mL). Ether (30 mL) was added slowly at 0 °C, and the resulting precipitates were collected by filtration, washed with ether, and dried to give 16 g (57%) of 1-(2-bromoethyl)imidazole hydrogen bromide as a pale brown powder: mp 105–108 °C; NMR (DMSO-d_6) δ 4.00 (2H, d, J = 7 Hz), 4.72 (2H, d, J = 7 Hz), 7.70–8.02 (2H, m), 9.38 (1H, bs). A mixture of 4 (2 g, 9 mmol), 1-(2-bromoethyl)imidazole hydrogen bromide (2.5 g, 10 mmol), and K_2CO_3 (4.4 g, 24 mmol) in DMF (100 mL) was stirred for 5 h at 80 °C. The mixture was cooled, and 2 N HCl and AcOEt were added to the mixture. The acidic aqueous layer was separated, made alkaline with 5% K_2CO_3 solution, and extracted with AcOEt (300 mL). The extract was washed with brine, dried, and concentrated under reduced pressure. The residual solids were purified by chromatography on silica gel with $CHCl_3$–MeOH (20:1) and recrystallized from AcOEt–hexane to give 2.3 g (82%) of 5a as white crystals. The analytical data of compound 5a obtained by method II agreed with those of compound 5a prepared by method I.

Preparation of 2-[2-(1-Imidazolyl)ethyl]-4-methyl-1(2*H*)-phthalazinone (9a). Treatment of 7a with 80% hydrazine hydrate, as described above, gave 8a (89%) as white crystals: mp 219–221 °C (EtOH). Introduction of an imidazolylethyl group was performed as described above (method II) to give 9a: yield 72%; mp 89–90 °C (AcOEt–hexane); IR (KBr) 1645 cm^{-1}; NMR ($CDCl_3$) δ 2.50 (3H, s), 4.30–4.58 (4H, m), 6.85–7.02 (2H, m), 7.36 (1H, bs), 7.60–7.77 (3H, s), 8.23–8.50 (1H, m).

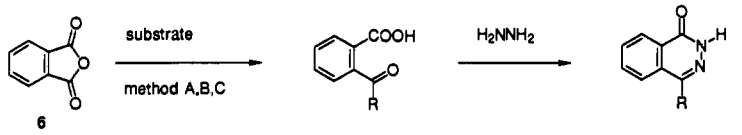
By the same procedure, 12a–d were prepared from commercially available 10a–d, and these are summarized in Tables III and VII.

Preparation of 2-[2-(1-Imidazolyl)ethyl]-4-[2-(5-ethylthienyl)]-1(2*H*)-phthalazinone (15f) (Method A). To a suspension of 6 (10 g, 67.5 mmol) in CH_2Cl_2 (150 mL) was added $AlCl_3$ (10.7 g, 80 mmol) in portions at 0 °C. A solution of 2-ethylthiophene (8.9 g, 79.4 mmol) in CH_2Cl_2 (30 mL) was added, and the mixture was stirred overnight at room temperature. The mixture was poured into diluted HCl solution and extracted with 400 mL of $CHCl_3$. The extract was shaken with 5% K_2CO_3, and the alkaline washings were made acidic with diluted HCl solution and extracted with $CHCl_3$. The extract was dried and concentrated under reduced pressure. The residual solids were recrystallized from $CHCl_3$–hexane to give 10 g (57%) of 2-(5-ethylthienoyl)benzoic acid (13f) as pale pinkish crystals: mp 93–94 °C; IR (KBr) 1685, 1650 cm^{-1}; NMR ($CDCl_3$) δ 1.32 (3H, t, J = 7 Hz), 2.88 (2H, q, J = 7 Hz), 6.71 (1H, d, J = 4 Hz), 7.04 (1H, d, J = 4 Hz), 7.33–7.79 (4H, m), 7.91–8.17 (1H, m). A solution of 13f (5.0 g, 19 mmol) and 80% hydrazine hydrate (1.8 g, 29 mmol) in EtOH (200 mL) was refluxed for 5 h. After cooling, the resulting precipitates were filtered, washed with EtOH, and recrystallized from EtOH to give 4.3 g (89%) of 14f as white crystals: mp 191–192 °C; IR (KBr) 1660 cm^{-1}; NMR (DMSO-d_6) δ 1.32 (3H, t, J = 7 Hz), 2.95 (2H, q, J = 7 Hz), 7.02 (1H, d, J = 4 Hz), 7.43 (1H, d, J = 4 Hz), 7.87–8.48 (4H, m), 12.70 (1H, bs). Treatment of 14f with 1-(2-bromoethyl)imidazole hydrogen bromide in the presence of K_2CO_3 (method II) gave 15f (73%): mp 110–112 °C; IR 1660 cm^{-1}; NMR ($CDCl_3$) δ 1.37 (3H, t, J = 7 Hz), 2.90 (2H, q, J = 7 Hz), 4.40–4.72 (4H, m), 6.71–8.22 (8H, m), 8.29–8.60 (1H, m).

In a similar manner, 12e, 12h–j, 12l, 15a, 15c–e, and 15g were prepared from 6, and these are summarized in Tables III and VII.

Preparation of 2-[2-(1-Imidazolyl)ethyl]-4-(3,4-dimethoxyphenyl)-1(2*H*)-phthalazinone (12k). Treatment of 6 with catechol in the presence of $AlCl_3$ afforded crude 2-(3,4-dihydroxybenzoyl)benzoic acid as solids. To a solution of these solids in DMF were added iodomethane (5 molar equiv) and K_2CO_3 (5 molar equiv), and the mixture was stirred overnight at room

Table VII. Preparation of Acylbenzoic Acid and Its Cyclization



substrate	method[a]	no.	yield (%)	mp, °C (recrystn solvent)[b]	no.	yield (%)	mp, °C (recrystn solvent)[b]
		3[c]			**4**	92	233–235[d] (F)
		7a[c]			**8a**	89	219–221[e] (G)
isobutyl bromide	B	**7b**	crude[f]		**8b**	13[g]	141–143 (B)
C_5H_9MgCl[c]	B	**7c**	crude[f]		**8c**	11[g]	156–157 (B)
$C_6H_{11}MgCl$[c]	B	**7d**	crude[f]		**8d**	9[g]	174–175 (B)
		10a[c]			**11a**	86	252–253 (G)
		10b[c]			**11b**	88	272–273 (G)
		10c[c]			**11c**	91	258–259[h] (G)
		10d[c]			**11d**	89	248–250 (G)
i-Pr-PhH	A	**10e**	68	123–124 (A)	**11e**	79	262–263 (G)
3-CF_3-PhBr	B	**10f**	67	165–167[i] (B)	**11f**	89	218–220 (F)
4-CF_3-PhBr	B	**10g**	56	176–178[j] (B)	**11g**	73	>300 (F)
MeO-PhH	A	**10h**	36	141–142 (A)	**11h**	82	241–242 (G)
EtO-PhH	A	**10i**	45	109–111 (A)	**11i**	74	253–255 (G)
MeS-PhH	A	**10j**	52	149–151 (A)	**11j**	72	250–251 (G)
1,2(MeO)$_2$-PhH	A[k]	**10k**	47[l]	oil[l]	**11k**	53	251–252 (G)
F-2-MeO-PhH	A	**10l**	55	177–179 (B)	**11l**	72	>300 (F)
thiophene	A	**13a**	72	145–147[m] (C)	**14a**	79	193–195 (G)
furan	C	**13b**	43	150–152[n] (A)	**14b**	72	151–153 (G)
2-Cl-thiophene	A	**13c**	67	107–109 (A)	**14c**	80	183–185 (F)
2-Me-thiophene	A	**13d**	78	134–135 (C)	**14d**	88	190–192 (G)
3-Et-thiophene	A	**13e**	51	160–162 (C)	**14e**	71	172–173 (G)
2-Et-thiophene	A	**13f**	57	93–94 (D)	**14f**	89	191–192 (G)
2-MeO-thiophene	A	**13g**	31	157–159 (C)	**14g**	35	182–184 (F)
2-Ac-thiophene[o]	C	**13h**[p]	61	158–159[p] (A)	**14h**[p]	86	222–223[p] (B)
2-bromopyridine	C	**16a**	38	215–217 (E)	**17a**	80	233–234 (G)
3-bromopyridine	C	**16b**	47	161–162 (E)	**17b**	90	269–270 (F)
4-bromopyridine	C	**16c**	32	252–253 (E)	**17c**	83	293–294 (G)

[a] See footnote *a* in Scheme II. [b] Recrystallization solvent: (A) AcOEt–hexane; (B) AcOEt–EtOH; (C) AcOEt; (D) $CHCl_3$–hexane; (E) AcOEt–MeOH; (F) EtOH–DMF; (G) EtOH. [c] Commercially available. [d] Literature[18] 232–234 °C. [e] Literature[19] 219–220 °C. [f] We failed to isolate **7b–d** in purified form. [g] Yield from **6**. [h] Literature[18] 259–260 °C. [i] Literature[15] 164–166 °C. [j] Literature[15] 176–178 °C. [k] After the reaction by method A, obtained compound was converted into (3,4-dimethoxybenzoyl)benzoic acid methyl ester by the reaction with MeI in the presence of K_2CO_3. [l] As methyl ester. [m] Literature[20] 146–147 °C. [n] Literature[21] 152–153 °C. [o] As ethylene acetal [mp 32–33 °C (lit.[22] 32–33 °C)]. [p] As ethylene acetal.

temperature. The reaction mixture was concentrated, extracted with AcOEt, and dried. The residue from the extract was chromatographed on silica gel with $CHCl_3$ to give methyl 2-(3,4-dimethoxybenzoyl)benzoate (**10k**) (47% from **6**) as oil: NMR ($CDCl_3$) δ 3.80–3.92 (9H, m), 6.75–8.03 (7H, m); MS *m/e* 300 (M^+, 1) 162 (100). Conversion of **10k** to **12k** through **11k** was performed as described above.

Preparation of 2-[2-(1-Imidazolyl)ethyl]-4-cyclohexyl-1(2*H*)-phthalazinone (9d) (Method B). To a solution of **6** (30 g, 0.2 mol) in THF (250 mL) was added dropwise at 15 °C under a nitrogen atmosphere a 2.0 M solution of cyclohexylmagnesium chloride (in 100 mL THF, 0.2 mol). After stirring for 30 min at room temperature, the mixture was poured into ice-water and concentrated. To the residual oil, AcOEt and 1% K_2CO_3 solution were added, and the alkaline aqueous layer was separated. The aqueous layer was made acidic with diluted HCl solution and extracted with AcOEt (500 mL). The extract was washed with brine, dried, and concentrated under reduced pressure. The residue was chromatographed on silica gel with $CHCl_3$–MeOH (20:1) to give **7d** (6 g, 13%) as pale yellow solids: IR (KBr) 1750 cm^{-1}; NMR ($CDCl_3$) δ 0.90–2.60 (11H, m), 6.52 (1H, bs), 7.21–7.86 (4H, m). Treatment of **7d** with 80% hydrazine hydrate, as described above, gave **8d** (66%) as white crystals: mp 174–175 °C (AcOEt–EtOH); IR (KBr) 1660 cm^{-1}; NMR ($CDCl_3$) δ 1.10–2.30 (10H, m), 2.80–3.42 (1H, m), 7.45–7.93 (3H, m), 8.30–8.62 (1H, m), 12.2 (1H, bs). Treatment of **8d** with 1-(2-bromoethyl)-imidazole in the presence of K_2CO_3 gave **9d** (45%): mp 151–153 °C ($CHCl_3$–hexane); IR (KBr) 1640 cm^{-1}; NMR ($CDCl_3$) δ 1.02–2.25 (10H, m), 2.72–3.40 (1H, m), 4.30–4.72 (4H, m), 6.78–7.02 (2H, m), 7.27 (1H, s), 7.46–7.88 (3H, m), 8.17–8.45 (1H, m).

Via the literature procedure,[15] phthalic anhydride was allowed to react with the Grignard reagents prepared from 3-bromobenzotrifluoride and 4-bromobenzotrifluoride to give **10f** and **10g**. By the same procedure, **7b** and **7c** were prepared using the Grignard reagents obtained by the treatment of isobutyl bromide with magnesium and commercially available cyclopentylmagnesium chloride, respectively, and these are summarized in Tables II, III, and VII.

Preparation of 2-[2-(1-Imidazolyl)ethyl]-4-(3-pyridyl)-1(2*H*)-phthalazinone (18b) (Method C). To a solution of 1.6 M *n*-BuLi (in 78 mL hexane, 0.13 mol) in ether (150 mL) was added a solution of 3-bromopyridine (18.9 g, 0.12 mol) in ether (150 mL) dropwise at −78 °C under a nitrogen atmosphere, and the whole was stirred for 30 min at the same temperature. A solution of **6** (21.3 g, 0.14 mol) in THF (150 mL) was poured into the reaction mixture in one portion at −78 °C under nitrogen atmosphere, and the mixture was stirred for 1 h at the same temperature. After the mixture was allowed to warm to room temperature and quenched with water (300 mL), the alkaline aqueous layer was separated. The solution was adjusted to pH 4 with 6 N HCl, saturated with NaCl, and extracted with THF–AcOEt (500 mL). The extract was dried, concentrated, and chromatographed on silica gel with $CHCl_3$–MeOH (30:1) to give pale yellow solids. Recrystallization from AcOEt–MeOH gave **16b** (12.7 g, 47%) as white crystals: mp 161–162 °C; IR (KBr) 1690 cm^{-1}; NMR (DMSO-d_6) δ 7.33–8.15 (6H, m), 8.60–8.82 (2H,

m), 10.30 (1H, bs); MS *m/e* 227 (M^+, 3) 105 (100). Compound **17b** was prepared from **16b** by treatment with hydrazine hydrate as described above: yield 90%; mp 269–270 °C (EtOH–DMF); IR (KBr) 1680 cm^{-1}; NMR (DMSO-d_6) δ 7.32–9.00 (8H, m), 12.98 (1H, s); MS *m/e* 223 (M^+, 7) 221 (100). Compound **18b** was also prepared from **17b** as described above (method II): yield 57%; mp 147–148 °C (AcOEt–hexane); IR (KBr) 1650 cm^{-1}; NMR ($CDCl_3$) δ 4.41–4.60 (4H, m), 8.80–7.89 (8H, m), 8.20–8.50 (1H, m), 8.50–8.82 (2H, m); MS *m/e* 317 (M^+, 20) 95 (100).

By the same procedure, compounds **13b**, **13h** (as ethylene acetal derivatives), and **16a** and **16c** were obtained from **6** using lithiated reagents prepared from furan, 2-acetylthiophene ethylene acetal, 2-bromopyridine, and 4-bromopyridine by treatment with *n*-BuLi, respectively. Cyclization with hydrazine hydrate and introduction of a 2-(1-imidazolyl)ethyl group (method II) were performed as described above, and these are summarized in Table VII. Compound **14h** (an ethylene acetal derivative) was treated with (bromoethyl)imidazole in the presence of K_2CO_3, followed by treatment with diluted HCl solution to afford **15h**.

Preparation of 2-[2-(1-Imidazolyl)ethyl]-4-(3-carboxyphenyl)-1(2*H*)-phthalazinone (25b). To a solution of **19b** (13.7 g, 80 mmol) in THF (70 mL) was added dropwise at −78 °C under a nitrogen atmosphere a solution of 1.6 M *n*-BuLi (in 48 mL hexane, 80 mmol), and the mixture stirred for 30 min at the same temperature. A solution of *o*-tolunitrile (9.4 g, 80 mmol) in THF (20 mL) was added dropwise at −78 °C under a nitrogen atmosphere, and the whole was stirred for 1 h at the same temperature. The mixture was allowed to warm to room temperature, and diluted HCl was added. The acidic mixture was refluxed for 30 min and extracted with AcOEt. The extract was washed with brine, dried, concentrated, and chromatographed on silica gel with $CHCl_3$–hexane (1:2) to give 12.7 g (78%) of 2,3′-dimethylbenzophenone (**20b**) as a colorless oil: IR (neat) 1670 cm^{-1}; NMR ($CDCl_3$) δ 2.30 (3H, s), 2.34 (3H, s), 7.03–7.68 (8H, m). To a solution of **20b** (12.7 g, 60 mmol) in a mixture of pyridine (50 mL) and water (100 mL) was added a powder of $KMnO_4$ (120 g, 750 mmol) in portions at reflux, and the whole stirred for 2 h at the same temperature. The mixture was filtered and washed with MeOH, and the resulting filtrate was concentrated. The residual solids was dissolved in water (100 mL), and the solution was made acidic with diluted HCl. The resulting precipitates were collected by filtration, washed with water, and dried to give 13.5 g (83%) of **21b** as white solids: mp 262–265 °C; IR (KBr) 1685 cm^{-1}. A solution of **21b** (13 g, 59 mmol) and 80% hydrazine hydrate (4.2 g, 71 mmol) in EtOH (300 mL) was refluxed for 3 h. After cooling, diluted HCl was added to the mixture, and the resulting precipitates were collected, washed with EtOH, and dried to give 11 g (70%) of **22b** as white solids: mp >300 °C; IR (KBr) 1700, 1655 cm^{-1}; MS *m/e* 266 (M^+, 11) 263 (100). To a suspension of **22b** (1.7 g, 6.3 mmol) in EtOH (300 mL) was added concentrated H_2SO_4 (0.5 mL), and the mixture was refluxed for 12 h. After the solvent was evaporated, 5% K_2CO_3 solution (200 mL) was added, and the resulting precipitates were collected, dried, and recrystallized from EtOH–DMF to give 1.7 g (92%) of **23b** as white crystals: mp 212–213 °C; IR (KBr) 1735, 1695 cm^{-1}; NMR (DMSO-d_6) δ 1.36 (3H, t, J = 7 Hz), 4.34 (2H, q, J = 7 Hz), 7.52–8.50 (8H, m), 12.90 (1H, bs); MS *m/e* 294 (M^+, 15), 292 (100). Treatment of **23b** with 1-(2-bromoethyl)-imidazole in the presence of K_2CO_3 gave **24b**: yield 78%; mp 116–117 °C ($CHCl_3$–hexane); IR (KBr) 1730, 1650 cm^{-1}; NMR ($CDCl_3$) δ 1.39 (3H, t, J = 7 Hz), 2.43 (2H, q, J = 7 Hz), 4.30–4.75 (4H, m) 6.87–7.05 (2H, m) 7.29 (1H, bs), 7.37–7.85 (5H, m), 8.02–8.26 (2H, m) 8.30–8.59 (1H, m). A mixture of **24b** (1.5 g, 3.9 mmol) and 1 N NaOH (8 mL, 8 mmol) in EtOH (40 mL) was stirred for 24 h at room temperature. The mixture was adjusted to pH 7 with diluted HCl, and the resulting precipitates were collected by filtration, washed with EtOH, and dried to give 1.0 g (71%) of **25b** as white solids: mp 275–277 °C; IR (KBr) 1715, 1645 cm^{-1}; NMR (CF_3COOD) δ 4.73–5.12 (4H, m) 7.35–8.92 (11H, m).

By the same procedure, compounds **25a** and **25c** were prepared using 2-bromotoluene (**19a**) and commercially available 2,4′-dicarboxybenzophenone (**21c**) as the starting materials, respectively. Compound **30** was also prepared in a similar manner as described above using 2-(5-methylthienoyl)benzoic acid (**13d**) as the starting material, and these are summarized in Table VIII.

Table VIII. Carboxylic Acid and Its Ester Derivative

no.	yield (%)	mp, °C (recrystn solvent)[a]	no.	yield (%)	mp, °C (recrystn solvent)[a]
20a	54	oil	**22c**	89	>300 (solids)
21a	83	193–197 (solids)	**23c**	52	219–220 (F)
22a	66	>300 (solids)	**26**	52	265–268 (solids)
23a	61	205–206 (F)	**27**	65	>300 (solids)
			28	59	200–202 (F)

[a] See footnote *b* in Table VII.

Preparation of 2-[2-(1-Imidazolyl)ethyl]-4-(2-thienylmethyl)-1(2*H*)-phthalazinone (33c). A mixture of **6** (25 g, 0.375 mol), 2-thiopheneacetic acid (25 g, 0.175 mol), and $AcONa·H_2O$ (4 g, 0.029 mol) in dimethylacetoamide (20 mL) was refluxed for 4 h under a nitrogen atmosphere. A reaction mixture was poured into 0.1 N NaOH (200 mL) and extracted with AcOEt. The residue from the extract was dried, evaporated, and chromatographed on silica gel with $CHCl_3$–hexane (3:1) to give 9 g (22%) of **31c** as yellow solids: mp 112–114 °C; IR (KBr) 1765 cm^{-1}; NMR ($CDCl_3$) δ 6.55 (1H, s), 6.84–7.10 (1H, m) 7.18–7.96 (6H, m). A solution of **31c** (2.8 g, 12.2 mmol) and 80% hydrazine hydrate (0.9 g, 14.6 mmol) in EtOH (200 mL) was refluxed for 3 h. The mixture was adjusted to pH 7 with diluted HCl, and the resulting precipitates were collected by filtration, washed with water, and recrystallized from EtOH to give 2.8 g (95%) of **32c** as white crystals: mp 162–164 °C; IR (KBr) 1655 cm^{-1}; NMR ($CDCl_3$–CD_3OD (10:1)) δ 4.43 (2H, s), 6.78–7.22 (3H, m), 7.55–7.90 (3H, m), 8.27–8.52 (1H, m). Introduction of a 2-(1-imidazolyl)ethyl group was performed as described above, and recrystallization from $CHCl_3$–hexane gave **33c** (42%) as white crystals: mp 135–137 °C; IR (KBr) 1655 cm^{-1}; NMR ($CDCl_3$) δ 4.32–4.78 (4H, m), 4.40 (2H, s) 6.72–7.44 (6H, m), 7.53–7.86 (3H, m), 8.20–8.52 (1H, m); MS *m/e* 336 (M^+, 11) 96 (100).

Via the literature procedure,[12] compound **6** was allowed to react with phenylacetic acid and 4-chlorophenylacetic acid in the presence of AcONa to give **31a** and **31b**, followed by treatment with hydrazine hydrate to afford **32a** and **32b**, respectively. 4-Benzyl-1(2*H*)-phthalazinone (**32a**): mp 198–200 °C (EtOH) (lit.[16] mp 197–199 °C). 4-(4-Chlorobenzyl)-1(2*H*)-phthalazinone (**32b**): mp 224–226 °C (EtOH) (lit.[9] mp 220–222 °C).

By the same procedure (method II) described above, **33a** and **33b** were prepared from **32a** and **32b**, respectively, and these are summarized in Table II.

***In Vitro* Enzyme Assay of TXA_2 Synthetase.** Rabbit platelet microsomes as the enzyme source were prepared according to the methods of Needleman.[17] 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, a reaction mixture was preincubated with the vehicle, and the 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.

***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 clotting 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.

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 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 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 ip 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 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 constriction (control response) was obtained. Test compound (10 mg/kg) was administered iv 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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