Chem. Pharm. Bull. **42**(3) 560—569 (1994) Vol. 42, No. 3

Studies on Anti-platelet Agents. II.[1] Synthesis and Platelet-Inhibitory Activity of 5-Methyl-4-(3-pyridyl)-2-(substituted Benzimidazol-5-yl)imidazoles[2]

Akito TANAKA,* Kiyotaka ITO, Shigetaka NISHINO, Yukio MOTOYAMA, and Hisashi TAKASUGI

New Drug Research Laboratories, Fujisawa Pharmaceutical Co., Ltd., 2–1–6 Kashima, Yodogawa-ku, Osaka 532, Japan. Received August 18, 1993; October 18, 1993

A series of 5-methyl-4-(3-pyridyl)-2-(substituted benzimidazol-5-yl)imidazole derivatives was synthesized and tested for anti-platelet and vasodilatory activities. Some compounds were found to have potent activities and low acute toxicity. In particular, 5-methyl-4-(3-pyridyl)-2-(7-chloro-6-methoxy-2-methylbenzimidazol-5-yl)imidazole (26) and 5-methyl-4-(3-pyridyl)-2-(7-chloro-3-methoxy-2-methylbenzimidazol-5-yl)imidazole (33) exhibited 63% or 51% inhibition at a dose of 10 mg/kg for anti-patelet activity *ex vivo* in rats, respectively, while they showed no toxicity even at 180 or 100 mg/kg, respectively. Compound 33 also exhibited potent vasodilatory activity (ED_{50} = 11 μg/ml). Enzyme studies on these imidazoles showed that the novel imidazoles inhibit some enzymes which are involved in the platelet aggregation cascade such as cyclooxygenase, phosphodiesterase (PDE), and thromboxane A_2 synthetase. The enzyme assay also suggested that the inhibitory activity on PDE may account for the vasodilatory activity of these imidazoles.

Keywords platelet aggregation; inhibitor; vasodilatory activity; 5-methyl-4-(3-pyridyl)-2-benzimidazolyl-imidazole; acute toxicity

The effectiveness of anti-platelet agents for the treatment of thrombotic disease has been conclusively shown during the past decade through laboratory and clinical studies.[3] However, these studies have also revealed deficiencies associated with currently available drugs which offer the clinician imprecise and limited control over platelet function and are characterized by a high incidence of side effects.[4]

In a previous paper,[1] we reported that 5-methyl-4-(3-pyridinyl)-2-(substituted phenyl)imidazoles (**1**) exhibited potent inhibitory activity on platelet aggregation in rat (*ex vivo* and *in vitro*) due to inhibition of some enzymes such as cyclooxygenase (CO), thromboxane A_2 (TXA_2) synthetase, and phosphodiesterase (PDE). In particular 2-(4-acetylamino-5-chloro-2-methoxyphenyl)-5-methyl-4-(3-pyridinyl)imidazole (**2**) exhibited potent anti-platelet activity *ex vivo* in rats (87.8% inhibition 1 h after administration of 32 mg/kg) with vasodilatory activity (ED_{50} = 66 μM). The vasodilatory activity of **2** is considered to be beneficial in thrombotic disease. Moreover, **2** demonstrated little acute toxicity and weak ulcerogenic activity in rat stomach (no effect up to 32 mg/kg), while use of aspirin, the most extensively used anti-platelet drug, is restricted by a high incidence of the side effect.[5]

In our continuing efforts to obtain useful anti-platelet agents, we have carried out further modification of the imidazole derivatives. A series of 2-(benzimidazol-5-yl)-5-methyl-4-(3-pyridyl)imidazoles (**3**) was synthesized and studied by means of several pharmacological assays since we considered compounds with a heterocyclic ring condensed at the 4 and 5 positions of the phenyl ring of **2** as bio-isosteres of the imidazoles (Chart 1). We describe here the syntheses of **3** and present pharmacological data on their anti-platelet activities (*ex vivo* and *in vitro*), enzyme-inhibitory activities (CO, TXA_2 synthetase, PDE) vasodilatory activity, and actue toxicity.

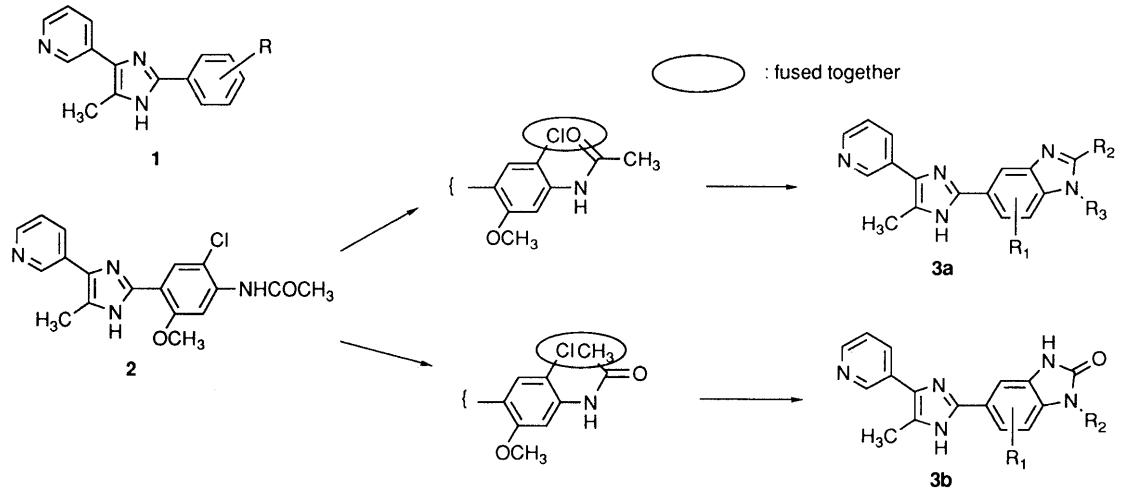
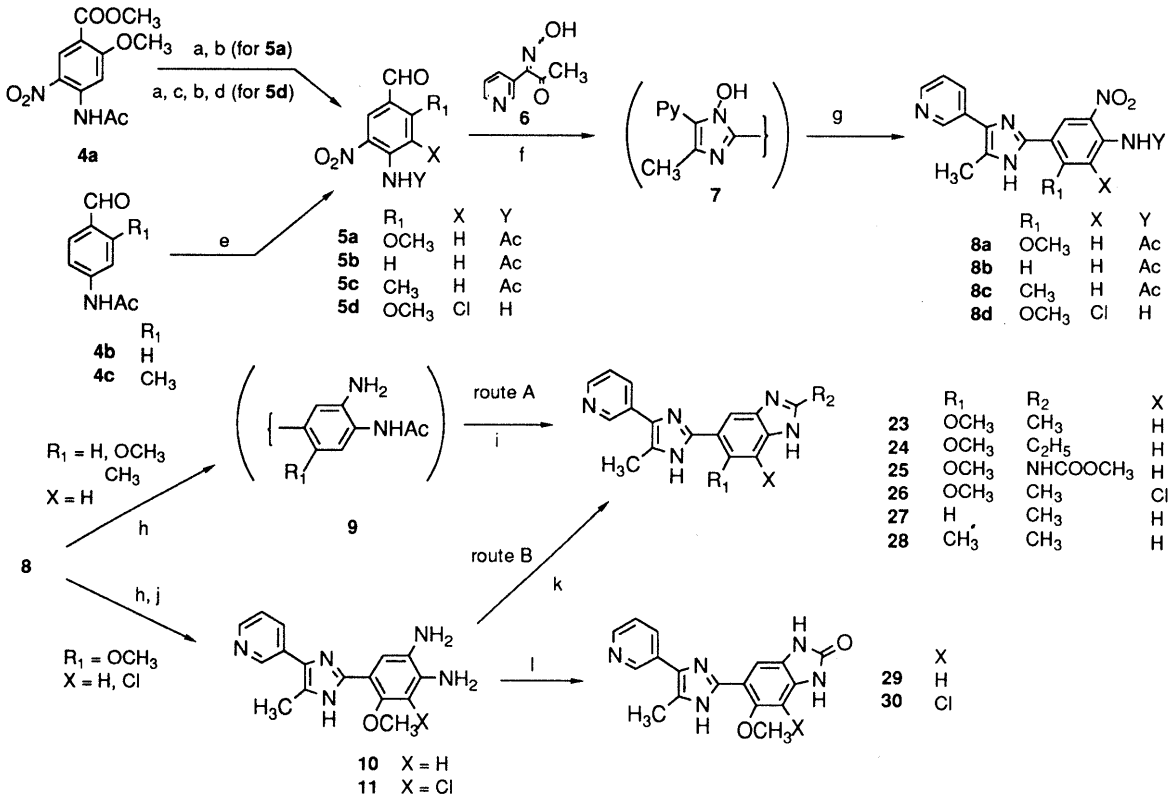


Chart 1

Chemistry

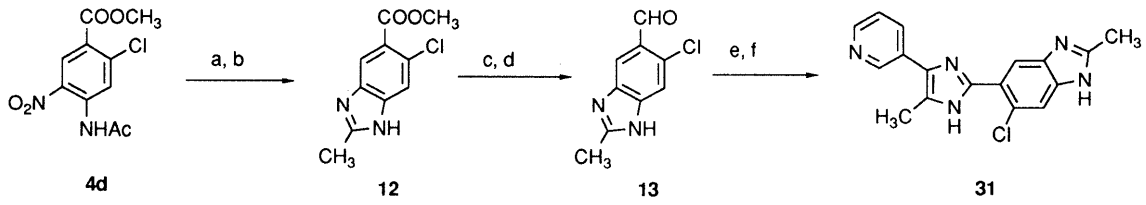
The synthetic methods leading to the imidazole and benzimidazole moieties were carried out according to literature procedures.[1,6]

The synthesis of 5-methyl-4-(3-pyridyl)-2-(2,6,7-substituted benzimidazol-5-yl)imidazole derivatives (**23**—**31**) is summarized in Charts 2 and 3. Reduction of methyl 4-acetylamino-2-methoxy-5-nitrobenoate (**4a**) with $LiAlH_4$ and subsequent oxidation with activated MnO_2 provided 4-acetylamino-2-methoxy-5-nitrobenzaldehyde (**5a**). Nitration of 4-acetylaminobenzaldehydes (**4b**, **4c**) gave the corresponding 4-acetylamino-5-nitrobenzaldehydes (**5b**, **5c**). Reduction of **4a** with $LiAlH_4$ and subsequent treatment with 4 N NaOH afforded 4-amino-2-methoxy-5-nitrobenzyl alcohol. Oxidation of the alcohol with activated MnO_2 provided 4-amino-2-methoxy-5-nitrobenzaldehyde, followed by reaction with a complex of Cl_2 and iodobenzene in a mixture of pyridine (2 eq) and tetrahydrofuran (THF) at room temperature to afford 4-amino-3-chloro-2-methoxy-5-nitrobenzaldehyde (**5d**). Condensation of **5a**—**d** with 1-hydroxyimino-1-(3-pyridyl)-2-propanone (**6**) and ammonium acetate in refluxing glacial acetic acid afforded 1-hydroxy-4-methyl-5-(3-pyridyl)-2-(substituted phenyl)imidazoles (**7**). The *N*-hydroxyimidazoles (**7**) were readily converted to the corresponding imidazoles (**8**) with $P(OEt)_3$ in dimethylformamide (DMF) at 50—60 °C.[1] Reduction of **8a**—**c** with Fe and NH_4Cl in refluxing ethanol (EtOH) afforded **9**. The 4-acetylamino-5-aminophenyl moiety of **9** was converted to the benzimidazole ring in refluxing acidic methanol (MeOH) to afford the required compounds (**23**, **27**, **28**, route A). Hydrolysis of the acetylamino moiety of **8a** and **8d** in 6 N HCl, and subsequent reduction with Fe and NH_4Cl gave 2-(4,5-diaminophenyl)-5-methyl-4-(3-pyridyl)imidazole derivatives (**10**, **11**), respectively. Condensation of **10** with propionic acid, 1,3-bis(methoxycar-



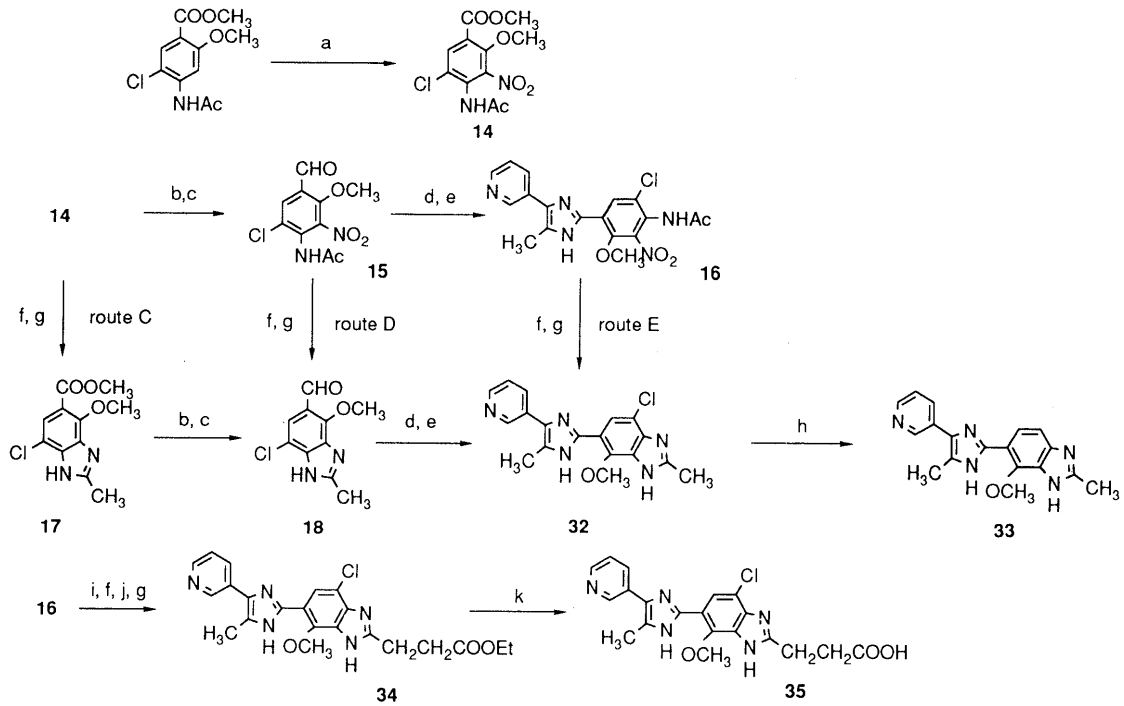
a) $LiAlH_4$; b) activated MnO_2; c) 4 N NaOH; d) Cl_2; e) HNO_3; f) **6**, $AcONH_4$ in AcOH, reflux; g) $P(OEt)_3$; h) Fe, NH_4Cl; i) H_2SO_4-MeOH, reflux; j) hydrolysis *via* H_3O^+ (except **8d**); k) propionic acid for **24**, acetic acid for **26**, 1,3-bis(methoxycarbonyl)-2-methylisothiourea for **25**; l) carbodiimidazole

Chart 2



a) Fe, NH_4Cl; b) H_2SO_4-MeOH, reflux; c) $LiAlH_4$; d) activated MnO_2; e) **6**, $AcONH_4$ in AcOH, reflux; f) $P(OEt)_3$

Chart 3



a) HNO_3; b) $LiAlH_4$; c) activated MnO_2; d) **6**, $AcONH_4$ in AcOH, reflux; e) $P(OEt)_3$; f) Fe, NH_4Cl; g) H_2SO_4-MeOH, reflux; h) H_2/Pd-C with NEt_3; i) hydrolysis *via* H_3O^+; j) $ClCOCH_2CH_2COOEt$; k) 6 N HCl aq.

Chart 4

bonyl)-2-methylisothiourea, and carbodiimidazole gave 2-ethyl-6-methoxybenzimidazol-5-yl (**24**, route B), 6-methoxy-2-methoxycarbonylaminobenzimidazol-5-yl (**25**, route B), and 6-methoxy-2-(3*H*)-benzimidazolon-5-yl derivatives (**29**), respectively. 2-(7-Chloro-6-methoxy-2-methylbenzimidazol-5-yl)-5-methyl-4-(3-pyridyl)imidazole (**26**) was prepared from **11** in a manner similar to that used to obtain **24**. Cyclization of **11** with carbodiimidazole provided 2-(7-chloro-6-methoxy-2(3*H*)-benzimidazolon-5-yl)-5-methyl-4-(3-pyridyl)imidazole (**30**).

The synthetic approach to 2-(6-chloro-2-methylbenzimidazol-5-yl)-5-methyl-4-(3-pyridyl)imidazole (**31**) was to convert methyl 4-acetylamino-2-chloro-5-nitrobenzoate (**4d**) into 6-chloro-5-methoxycarbonyl-2-methylbenzimidazole (**12**), followed by reduction of **12** with $LiAlH_4$ and subsequent oxidation with activated MnO_2 to obtain 6-chloro-5-formyl-2-methyl-benzimidazole (**13**). Compound **13** was condensed with **6** to give **31** (Chart 3).

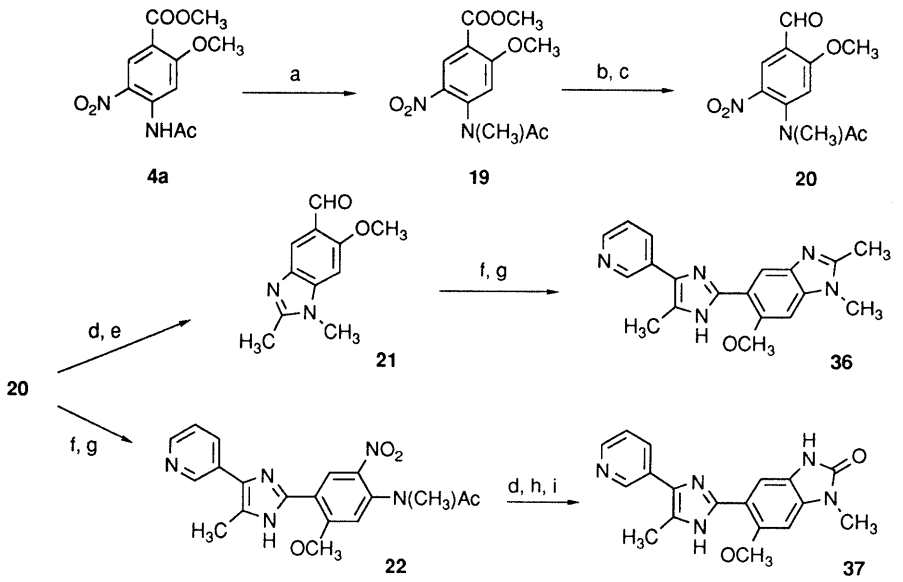
Synthesis of 2-(4-methoxy-2-alkylbenzimidazol-5-yl)-5-methyl-4-(3-pyridyl)imidazole derivatives (**32**, **33**) is summarized in Char 4. 2-(4-Chloro-7-methoxy-2-methylbenzimidazol-6-yl)-5-methyl-4-(3-pyridyl)imidazole (**32**) was synthesized from methyl 4-acetylamino-5-chloro-2-methoxy-3-nitrobenzoate (**14**) *via* three routes (routes C, D, E). These three routes consist of the same three steps, and each route differs from the others in the order of preparation of each moiety. For example, in route C, after the benzimidazole ring was constructed, the ester was converted to the aldehyde, followed by condensation reaction with **6** to give **32**. On the other hand, the benzimidazole ring was constructed at the final step in the route E. Overall yields of these routes from **14** to **32** are summarized in Table I. Among these routes, route D showed the highest overall yield (27.9%). Hydrogenation of **32** on 10% Pd–C in the presence of triethylamine in MeOH at room temperature afforded 2-(4-methoxy-2-methylbenzimidazol-5-yl)-5-methyl-4-(3-pyridyl)-imidazole (**33**). 2-[7-Chloro-2-(2-ethoxycarbonyl)ethyl-4-methoxybenzimidazol-5-yl]-5-methyl-4-(3-pyridyl)imidazole (**34**) was synthesized by hydrolysis of **16** with 6 N HCl to 2-(3,4-diamino-5-chloro-2-methoxyphenyl)-5-methyl-4-(3-pyridyl)imidazole, followed by reduction of the nitro moiety with Fe and ammonium chloride, acylation with ethyl 3-cholorocarbonyl propionate, and intramolecular cyclization. Hydrolysis of **34** in 6 N HCl led to 2-[2-(2-carboxy)ethyl-7-chloro-4-methoxybenzimidazol-5-yl]-5-methyl-4-(3-pyridyl)imidazole (**35**).

1-Methylbenzimidazoles (**36**, **37**) were synthesized from methyl 4-(*N*-acetyl-*N*-methylamino)-2-methoxy-5-nitrobenzoate (**19**, Chart 5). Compound **19** was prepared by *N*-methylation of **4a** with methyl iodide in the presence of NaH. Syntheses of 5-methyl-2-(6-methoxy-1,2-dimethylbenzimidazol-5-yl)-4-(3-pyridyl)imidazole (**36**) and 5-methyl-2-(6-methoxy-1-methyl-2(3*H*)-benzimidazolon-5-yl)-4-(3-pyridyl)imidazole (**37**) were carried out similarly to those of **23** and **29**, respectively.

Table I. Comparison of Total Yields[a] in Routes C, D, and E[b]

Route	(Paths[c])	Total yield (%)
C	(**14**–**17**–**18**–**32**)	17.4
D	(**14**–**15**–**18**–**32**)	27.9
E	(**14**–**15**–**16**–**32**)	24.4

a) Yields from **14** to **32**. *b*) See Chart 4. *c*) Bold numbers stand for the compound numbers in Chart 4.



a) CH_3I, NaH; b) $LiAlH_4$; c) activated MnO_2; d) Fe, NH_4Cl; e) H_2SO_4-MeOH, reflux; f) **6**, $AcONH_4$ in AcOH, reflux; g) $P(OEt)_3$ h) H_3O^+; i) carbodiimidazole

Chart 5

Results and Discussion

In order to assess the biological activity of the compounds discussed in this study, two assay systems were used: (i) platelet aggregation induced by collagen in rat *ex vivo* and *in vitro*; (ii) relaxation of KCl-contracted aorta from rat (vasodilatory activity). Some compounds considered to be promising were tested for inhibitory activities towards several enzymes involved in platelet aggregation cascade (CO, TXA_2 synthetase, and PDE) and for acute toxicity.

In the previous paper, we reported that **2** exhibited potent anti-platelet activity with vasodilatory activity, low toxicity, and weak ulcerogenesis. Further modification of **2** was carried out in our continuous efforts to obtain anti-platelet agents with clinical potential, since we thought the pharmacological properties of **2** appropriate for thrombotic disease. We presumed that compounds with an imidazole ring fused at the 4 and 5 positions of the phenyl ring of **2**, 2-(substituted 1*H*-benzimidazol-5-yl)-5-methyl-4-(3-pyridyl)imidazoles, are bio-isosters of **2** (Chart 1). There are two ways to condense the imidazole ring, as shown in Chart 1, that is, cyclization between the chlorine of the phenyl ring and oxygen or methyl of the acetyl moiety of **2** produces 2-(2-alkyl-substituted benzimidazol-5-yl)-5-methyl-4-(3-pyridyl)imidazole (**3a**) and 5-methyl-4-(3-pyridyl)-2-(substituted 2(3*H*)-benzimidazolon-5-yl)-imizazole (**3b**), respectively.

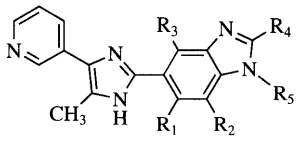
We first synthesized 2-(6-methoxy-2-methylbenzimidazol-5-yl)-5-methyl-4-(3-pyridyl)imidazole (**23**), a derivative of **3a** in Chart 1. Compound **23** inhibited both platelet aggregation (42% inhibition at 32 mg/kg for *ex vivo* study) and KCl-induced contraction ($ED_{50}=48\,\mu g/ml$) as expected (Table II). The potencies of **23** were insufficiently high so that modifications of **23** were carried out, and the pharmacological data obtained are listed in Table II.

Substitutions of the methyl group at the 2 position of the benzimidazole ring of **23** for an ethyl group (**24**) and of the methoxy at the 6 position for hydrogen (**27**), methyl group (**28**), and chloro substituent (**31**) decreased the anti-platelet activity. Introduction of a chloro substituent onto the 7 position of the benzimidazole ring provided **26**, with a large increase of the anti-platelet activity *ex vivo* (63% inhibition at 10 mg/kg). The *ex vivo* potency of **26** is three times more potent than that of the parent compound **23**, though its *in vitro* activity is three times less than that of **23**. Compound **26** showed little vasodilatory activity even at a dose of 100 μg/ml.

In order to study the effect of the position of the methoxyl group on the benzimidazole ring, 4-methoxybenzimidazol-5-yl (**32**) was synthesized. Compound **32** showed a large increase of anti-platelet activity *in vitro* ($IC_{50}=0.23\,\mu g/ml$) compared to **23**, but **32** was still equipotent to **23** in an *ex vivo* study. Introduction of a chloro substituent onto the benzimidazole ring of **32** provided **33** with potent anti-platelet activity (51% inhibition in the *ex vivo* study at a dose of 10 mg/kg), which is comparable with that of compound **26**. Moreover **33** demonstrated not only potent anti-platelet activity but also potent vasodilatory activity ($ED_{50}=11\,\mu g/ml$) while **26** showed little vasodilatory activity. Introduction of functional groups (**25**, **34**, **35**) at position 2 of the benzimidazole ring was found to lower anti-platelet activity. Replacement of hydrogen at position 1 of the benzimidazole ring with methyl (**36**) was carried out to study the effect of tautomers of the imidazole moiety, but there was little effect on the anti-platelet activity *ex vivo* compared with **23**.

Second, synthesis of 2(3*H*)-benzimidazolone derivatives, **3b** in Chart 1, was carried out (Table II). 5-Methyl-4-(3-pyridyl)-2-[2(3*H*)-benzimidazolon-5-yl]imidazole (**29**) exhibited potent anti-platelet activity *in vitro* ($IC_{50}=0.84\,\mu g/ml$) and had the most potent vasodilation

TABLE II. 5-Methyl-4-(3-pyridyl)-2-(substituted benzimidazol-5-yl)imidazole Derivatives and Their Anti-platelet and Vasodilatory Activities



	R_1	R_2	R_3	R_4	R_5	Anti-platelet[a] *Ex vivo* (% inhibition)[b] dose (mg/kg) 32	10	3.2	Anti-platelet[a] *In vitro* collagen in rabbits IC_{50} (μg/ml)	Vasodilation[a] *In vitro* rat aorta ED_{50} (μg/ml)
Compound **3a**[c]										
23	OCH_3	H	H	CH_3	H	42	21		21	48
24	OCH_3	H	H	CH_2CH_3	H		8		NT	NT
25	OCH_3	H	H	$NHCOOCH_3$	H		NT		19	>100
26	OCH_3	Cl	H	CH_3	H	80	63	14	65	>100
27	H	H	H	CH_3	H		NT		44	19
28	CH_3	H	H	CH_3	H		18		46	46
31	Cl	H	H	CH_3	H		15		33	50
32	H	H	OCH_3	CH_3	H		16		0.23	49
33	H	Cl	OCH_3	CH_3	H	68	51		16	11
34	H	Cl	OCH_3	CH_2CH_2COOEt	H		6		NT	NT
35	H	Cl	OCH_3	CH_2CH_2COOH	H		6		NT	NT
36	OCH_3	H	H	CH_3	CH_3		22		NT	NT
Compound **3b**[c]										
29	OCH_3	H	H	>=O	H		NT[d]		0.84	0.22
30	OCH_3	Cl	H	>=O	H		17		2.5	NT
37	OCH_3	H	H	>=O	CH_3		11		NT	NT

a) The evaluation methods are described in the experimental section. *b*) *Ex vivo* activities were measured in rat 1 h after oral administration of each dose of compounds. *c*) See Chart 1. *d*) Solubility of **29** was too low to carry out an *ex vivo* study. NT: not tested.

TABLE III. Pharmacological Properties[a] of **23**, **26**, **29**, **32**, and Aspirin

Compound	Inhibitory activities IC_{50} (μg/ml) PDE[b]	CO[b]	TXA_2[b]	Acute toxicity in rat Dose (mg/kg)	Dead/sample
23	26	0.08	0.018	100	0/5
				180	0/5
26	8.0	10	NT	180	0/5
29	0.14	2.6	0.036	NT	
33	1.9	23	NT	100	0/5
				180	5/5
Aspirin	>18	40	>100	NT	

a) The evaluation methods are described in the experimental section. *b*) PDE: phosphodiesterase, CO: cyclooxygenase, TXA_2: thromboxane A_2 synthetase. NT: not tested.

activity of any compound in this study (ED_{50}=0.22 μg/ml). These activities were 25 and 220 times more potent than that of the parent compound **23**, respectively. Unfortunately, the solubility of **29** was so poor that we could not conduct an *ex vivo* study. We hypothesized that the insolubility of **29** was due to the strong hydrogen-bonding of the urea moiety of the 2(3*H*)-benzimidazolone ring; thus we introduced substituents (Cl: **30**, and CH_3: **37**) which we thought would lessen the hydrogen-bonding ability of the urea. These modifications increased the solubility, as expected, permitting *ex vivo* studies. However, the *ex vivo* potencies of **30** and **37** were slightly less than that of **23** while the *in vitro* potency of **30** was 10 times stronger than that of **23**.

Finally, the parent compound **23** and compounds which were potent in *in vitro* (**29**) and *ex vivo* studies (**26**, **33**) were selected for detailed phamacological tests. These results are summarized in Table III.

All of these novel imidazoles exhibited inhibitory activities on CO, PDE, and TXA_2 synthetase, while aspirin inhibited only CO. The inhibitory activity of these imidazoles on CO is much potent than that of aspirin. The TXA_2 synthetase inhibitory activiries of **23** and **29** were very potent (IC_{50}=0.018 and 0.036 μg/ml, respectively). Interestingly, the PDE-inhibitory activity of **29** was 190 times more poent than that of **23** and was consistent with the difference (220 times) in vasodilatory activity between **23** and **29**. These results suggested that the vasodilatory activity of these novel imidazoles may due to PDE inhibition and that the anti-platelet activity may due to a combination of PDE, CO, and TXA_2 synthetase inhibitons. The most potent compounds (**26**, **33**) in the *ex vivo* studies showed similar enzyme-inhibitory activities to **23**, while **26** and **33** exhibited *ca.* 3 times more potent *ex vivo* activity than **23**. While the reason for the difference between *in vitro* and *ex vivo* results is not clear at the moment, we thought that the reason may be that modifications of the benzimidazole ring influence either absorption, distribution, and metabolism, or a combination of these factors. Acute toxicities of all these compounds were very low, as shown in Table II. In particular, **26**, the most potent compound in the *ex vivo* study, exhibited no toxicity even at a dosage of 180 mg/kg. Further modifications and detailed pharmacological tests are being performed.

Experimental

Melting point determinations were performed in a capillary melting point apparatus (Thomas Hoover), without correction. The structures of all compounds were supported by their infrared (IR) (Hitachi 260-10) and 60 and 90 MHz proton nuclear magnetic resonance (^{1}H-NMR) (JEOL PMX-60SI and Varian EM-390) spectra. The mass spectra were measured with a Hitachi M-80 mass spectrometer. All compounds were analyzed for C, H, N, and the results were within 0.4% of the calculated theoretical values. No attempt was made to maximize the yields.

4-Acetylamino-2-methoxy-5-nitrobenzaldehyde (5a) Compound **4a** (37.43 g, 0.140 mol) was added in portions to a stirred mixture of $LiAlH_4$ (5.30 g, 0.140 mol) in THF (500 ml) at −30 °C, and the mixture was stirred at the same temperature for 2 h. The reaction mixture was allowed to come to room temperature, and then poured into a mixture of water (1000 ml) and ethyl acetate (AcOEt, 1000 ml). The whole mixture was adjusted to pH 1.8 with 10% HCl, and the resulting substance was removed by filtration. The separated organic layer was washed with water and brine, dried over $MgSO_4$, and concentrated to about one-tenth of the initial volume *in vacuo*. Ether (500 ml) was added to the mixture. The resulting crystalline precipitate was collected by filtration, washed with diethyl ether (Et_2O), and dried to give 4-acetylamino-2-methoxy-5-nitrobenzyl alcohol (14.70 g, 43.8%). IR (Nujol): 3450, 3350, 1690, 1620, 1580, 1500 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 2.20 (3H, s, $COCH_3$), 3.95 (3H, s, OCH_3), 4.50 (2H, s, CH_2OH), 5.32 (1H, br s, OH), 7.75 (1H, s), 8.15 (1H, s), 10.40 (1H, s, NH). A mixture of the above alcohol (14.64 g, 60.9 mmol) and activated MnO_2 (65.88 g, 4.5 w/w) in AcOEt (400 ml) was stirred and refluxed for 6 h. The insoluble substance was filtered off and washed with AcOEt. The filtrate was evaporated *in vacuo*, and the resulting crystalline precipitate was collected by filtration and washed with isopropyl ether (IPE) to give **5a** (10.92 g, 32.9%). Analytical data are given in Table V.

Compounds **13**, **15**, **18**, and **20** were prepared in a manner similar to that used to obtain **5a**, and the analytical data are summarized in Tables V and VI.

4-Acetylamino-3-nitrobenzaldehyde (5b) 4-Acetylaminobenzaldehyde (32.6 g, 0.2 mol) was added to nitric acid (d=1.52, 120 ml) in portions at −30—−40 °C. The reaction mixture was stirred at −20—−40 °C for 40 min, and then poured into a mixture of ice and water. To this mixture, AcOEt and THF were added, and the whole was adjusted to pH 7.5 with 20% K_2CO_3. The organic layer was washed with water and brine, and dried over $MgSO_4$. After removal of $MgSO_4$, the organic mixture was evaporated *in vacuo*. The resulting precipitate was washed with AcOEt and ether to give **5b** (20.9 g, 50.2%).

Compound **5c** was obtained similarly, and the analytical data are summarized in Table V.

4-Amino-3-chloro-2-methoxy-5-nitrobenzaldehyde (5d) Compound **4a** (91.5 g, 0.341 mol) was added to a mixture of $LiAlH_4$ (19.42 g, 0.512 mol) and THF (200 ml) in portions over 30 min at −45 °C. To the reaction mixture, AcOEt (20 ml), water (20 ml), 4 N NaOH (20 ml) and water (20 ml) were added successively after the end of the reduction had been confirmed by thin layer chromatography (TLC, silica gel, benzene: AcOEt = 4 : 1). The resulting residue was removed by filtration, and then the filtrate was evaporated *in vacuo* to give 4-amino-2-methoxy-5-nitrobenzyl alcohol. IR (Nujol): 3400, 3300, 1620, 1560 cm^{-1}. The alcohol was used in the following reaction without purification. A mixture of the alcohol and activated MnO_2 (348 g, 4 w/w) in AcOEt was stirred and refluxed for 4.5 h. After filtration, the filtrate was evaporated *in vacuo*. The resulting cystalline precipitate was colleced by filtration, and washed with Et_2O to give 4-amino-2-methoxy-5-nitrobenzaldehyde (33.26 g, 38.6%). ^{1}H-NMR (DMSO-d_6) δ: 3.80 (3H, s, OCH_3), 6.45 (1H, s, aromatic), 7.80 (2H, br s, NH_2), 8.28 (1H, s, aromatic), 9.90 (1H, s, CHO).

To an ice-cooled solution of the aldehyde (20.0 g, 0.102 mol) and dry pyridine (16.43 ml) in THF (400 ml) was added the crystalline precipitate of a complex of chlorine and iodobenzene (1 : 1)[7] (56.06 g, 0.204 mol), and the whole mixture was stirred at room temperature for 1 h. The reaction mixture was poured into a mixture of water (500 ml) and AcOEt (200 ml), and the separated organic layer was washed with water and dried over $MgSO_4$. After evaporation *in vacuo*, the resulting crystalline precipitate was collected by filtration and washed with IPE to give **5d** (9.14 g, 38.9%). IR (Nujol): 3450, 3350, 3300, 1680, 1650, 1600 cm^{-1}. MS m/z: 230 (M^+). Other analytical data of **5d** are given in Table V.

2-(4-Acetylamino-2-methoxy-5-nitrophenyl)-5-methyl-4-(3-pyridyl)-imidazole (8a) A mixture of **5a** (10.69 g, 44.9 mmol), 1-hydroxy-1-(3-pyridyl)-2-propanone (**6**, 7.02 g, 42.7 mmol), and ammonium acetate (32.94 g, 427 mmol) in acetic acid (70 ml) was stirred at 100 °C for 15 min. After removal of the solvent *in vacuo*, water (200 ml) and AcOEt (200 ml) were added, and the whole mixture was adjusted to pH 0.3 with 10% HCl. The aqueous phase was washed once with AcOEt and neutralized with K_2CO_3. The resulting precipitate was collected, washed with water and AcOEt, and dried to give 2-(4-acetylamino-2-methoxy-5-nitrophenyl)-1-hydroxy-4-methyl-5-(3-pyridyl)imidazole (13.06 g, 79.8%), which was used for the next deoxygenation reaction without further purification. A mixture of the *N*-hydroxyimidazole (13.06 g, 34.1 mmol) and triethyl phosphite (14.03 ml, 81.8 mmol) in DMF (100 ml) was stirred at 80 °C. After cooling to room temperature, the mixture was poured into water (800 ml), and made alkaline with a small amount of K_2CO_3. The resulting precipitate was collected, washed with water, dried, and recrystallized from EtOH to give **8a** (9.49 g, 75.8%). Other analytical data of **8a** are given in Table VII.

Compounds **8b—d**, **16**, **22**, **31**, **32**, and **36** were prepared in a manner similar to that used in the case of **8a**, and the analytical data are summarized in Tables IV and VII.

Methyl 4-Acetylamino-5-chloro-2-methoxy-3-nitrobenzoate (14) Methyl 4-acetylamino-5-chloro-2-methoxybenzoate (130 g, 0.506 mol) was added to fuming HNO_3 (d=1.52, 330 ml) in portions over 10 min at −35—−20 °C, and the reaction mixture was stirred at −35—30 °C for 10 min, then poured into ice and water (3000 ml). The resulting precipitate was dissolved with AcOEt (1500 ml) and water (500 ml). The organic layer was washed with water and brine, and dried over $MgSO_4$. After filtration, the filtrate was evaporated *in vacuo*. The resulting precipitate was washed with IPE to give **14** (143 g, 93.8%), mp 129—131 °C. IR (Nujol): 3300, 1700, 1600, 1560, 1540, 1495 cm^{-1}. Other analytical data are given in Table V.

2-(6-Methoxy-2-methylbenzimidazol-5-yl)-5-methyl-4-(3-pyridyl)-imidazole (23) Compound **8a** (2.1 g, 5.7 mmol) was dissolved in methanol (MeOH, 150 ml) and THF (80 ml), and hydrogenated over 10% Pd–C (0.8 g) at room temperature under atmospheric pressure for 4 h. After filtration and evaporation of the filtrate, the resulting residue was dissolved in acetic acid (30 ml) and 6 N HCl (10 ml). The solution was stirred and refluxed for 2 h, and then poured into a mixture of water and AcOEt. The aqueous layer was washed with AcOEt, adjusted to pH 8 with 20% K_2CO_3, and then extracted with AcOEt. The organic layer was washed with water and brine, and dried over $MgSO_4$. After evaporation *in vacuo*, the residue was chromatographed (4% MeOH in $CHCl_3$, Al_2O_3) to give **23** (0.79 g, 43.4%). An analytical sample of **23** was prepared by recrystallization from ethanol (EtOH) and H_2O. IR (Nujol): 1640, 1600, 1560 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 2.50 (3H, s, CH_3), 2.53 (3H, s, CH_3), 4.00 (3H, s, OCH_3), 7.18 (1H, s), 7.45 (1H, dd, J=8, 5 Hz), 8.10 (1H, ddd, J=8, 2, 2 Hz), 8.13 (1H, s), 8.45 (1H, dd, J=5, 2 Hz), 8.97 (1H, d, J=2 Hz). Other analytical data of **23** are given in Table IV.

Other benzimidazole derivatives (**12**, **17**, **18**, **21**, **27—28**, **32**) were prepared in a manner similar to that of **23**, and the analytical data are summarized in Tables IV and VI.

2-(4,5-Diamino-2-methoxyphenyl)-5-methyl-4-(3-pyridyl)imidazole (10) A solution of **8a** (8.60 g, 23.4 mmol) in 6 N HCl (100 ml) was stirred at 85 °C for 2 h. The reaction mixture was allowed to cool to room temperature, and poured into a mixture of water (200 ml) and AcOEt (100 ml). The whole mixture was adjusted to pH 8 with 20% K_2CO_3, and the resulting precipitate was collected by filtration, washed with water, and dried to give 2-(4-amino-2-methoxy-5-nitrophenyl)-5-methyl-4-(3-pyridyl)imidazole (7.60 g, 100%), mp 245—248 °C (dec.). IR (Nujol): 3450, 3350, 3300, 3150, 1640, 1600, 1560, 1495 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 2.57 (3H, s, CH_3), 3.97 (3H, s, OCH_3), 6.38 (1H, s), 8.4—8.6 (2H, m), 8.8—9.4 (3H, m). A mixture of the above compound (3.25 g, 10 mmol), Fe (3.20 g, 53 mmol), and NH_4Cl (0.34 g, 6.3 mmol) in EtOH (150 ml) and water (20 ml) was vigorously stirred and refluxed for 3.5 h. After filtration to remove the insoluble material, which was washed with 50% MeOH in $CHCl_3$, the filtrate and washing were combined and dried over $MgSO_4$ and evaporated *in vacuo*. The resulting crystalline solid was washed with Et_2O and dried to give **10** (2.50 g, 84.7%), mp 122—125 °C (dec.). IR (Nujol): 3150, 1630, 1580, 1510 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 2.57 (3H, s), 3.90 (3H, s), 6.4 (1H, s), 7.40 (1H, s), 7.47 (1H, dd, J=8, 5 Hz), 8.10 (1H, ddd, J=8, 2, 2 Hz), 8.55 (1H, dd, J=5, 2 Hz), 8.93 (1H, d, J=2 Hz).

Compound **11** was prepared in a similar manner to that described for **10**, and its analytical data are summarized in Table VII.

Methyl 4-(*N*-Acetyl-*N*-methylamino)-2-methoxy-5-nitrobenzoate (19) Sodium hydride (1.3 g, 53.31 mmol) was gradually added over 20 min to an ice-cooled mixture of **4a** (14.3 g, 53.31 mmol) and DMF (200 ml), and the whole was stirred at room temperature for 1 h. It was cooled to 0 °C, then methyl iodide (3.7 ml, 58.64 mmol) was added. The reaction mixture was stirred at room temperature for 2 h, poured into a mixture of water and AcOEt, and adjusted to pH 3 with 10% HCl. The separated organic layer was washed with brine, dried over $MgSO_4$, and evaporated *in vacuo*. The resulting precipitate was recrystallized from Et_2O and IPE to give

19 (13.22 g, 87,8%). Analytical data of **19** are given in Table V.

2-(2-Ethyl-6-methoxybenzimidazol-5-yl)-5-methyl-4-(3-pyridyl)imidazole (24) A mixture of **10** (0.41 g, 1.38 mmol) and propionic acid (1.8 ml, 24.8 mmol) in concentrated HCl (10 ml) was stirred and refluxed for 14 h. After cooling to room temperature, the mixture was poured into a mixture of water (5 ml) and $CHCl_3$ (50 ml) and adjusted to pH = 9.5 with 4 N NaOH. After removal of resulting insoluble material, the separated organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The resulting precipitate was recrystallized from EtOH–Et_2O to give **24** (0.21 g, 45.6%). Analytical data of **24** are summarized in Table IV.

Compound **26** was prepared in a manner similar to that used for **24**, and its analytical data are given in Table IV.

2-(6-Methoxy-2(3*H*)-benzimidazolon-5-yl)-5-methyl-4-(3-pyridyl)-imidazole (29) A mixture of **10** (1.2 g, 4 mmol) and carbonyldiimidazole (1.0 g, 6 mmol) in DMF (20 ml) was stirred at room temperature for 6 h. The reaction mixture was poured into water. The resulting precipitate was collected by filtration and washed with water. The precipitate was dissolved with acidic water (pH 1) which was prepared by addition of 1 N HCl to water, and the acidic aqueous solution was washed with AcOEt. The aqueous phase was adjusted to pH 8 with 20% K_2CO_3, and extracted with 30% MeOH in $CHCl_3$. The separated organic layer was dried over $MgSO_4$ and evaporated *in vacuo*. The resulting precipitate was collected by filtration, and recrystallized from EtOH and water to give **29** (0.38 g, 29.7%). IR (Nujol): 3350, 1740, 1700, 1640, 1560 cm^{-1}. ^{1}H-NMR (D_2O–DCl) δ: 2.57 (3H, s), 3.93 (3H, s), 6.63 (1H, s), 7.13 (1H, s), 8.38 (1H, dd, J=8, 5 Hz), 8.77—9.20 (3H, m). MS (*m/z*): 321 (M^+). Other analytical data of **29** are given in Table IV.

The 7-chloro derivative (**30**) was obtained from **11** in a manner similar to that described for **29**. 2-(6-Methoxy-1-methyl-2(3*H*)-benzimidazolon-5-yl)-5-methyl-4-(3-pyridyl)-imidazole (**37**) was also obtained similarly from **22**.

2-(6-Methoxy-2-methoxycarbonylaminobenzimidazol-5-yl)-5-methyl-4-(3-pyridyl)imidazole (25) A 25% aqueous solution of NaOH was added to an ice-cooled stirred mixture of 2-methylthiopseudourea sulfate (1.40 g, 5 mmol) and methyl chloroformate (0.95 g, 10 mmol) in water (5 ml), until the pH of the reaction mixture reached 8. Care was taken to keep the temperature below 10—15 °C. The pH of the reaction mixture was then adjusted to 5 with glacial acetic acid (AcOH). The above mixture was added to a stirred mixture of **9** (1.5 g, 5 mmol) in EtOH (20 ml), and the whole was stirred at room temperature for 7 h, then poured into a mixture of AcOEt and water, and adjusted to pH 1 with 10% HCl. The aqueous phase was washed with AcOEt, neutralized with 20% K_2CO_3, and extracted with 10% MeOH in $CHCl_3$. The separated organic layer was dried over $MgSO_4$ and evaporated *in vacuo*. The residue was purified by Al_2O_3 gel column chromatography (2% MeOH in $CHCl_3$), and the resulting product was recrystallized from EtOH and water to give **25** (0.37 g, 20.4%). IR (Nujol): 3300, 1700, 1620, 1590 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 2.47 (3H, s), 3.63 (3H, s), 3.87 (3H, s), 5.38 (1H, br s), 6.48 (1H, s), 7.40 (1H, dd, J=8, 5 Hz), 7.80 (1H, s), 8.07 (1H, ddd, J=8, 2, 2 Hz), 8.42 (1H, dd, J=5, 2 Hz), 8.90 (1H, d, J=2 Hz). Other analytical data of **25** are given in Table IV.

2-(7-Methoxy-2-methylbenzimidazol-6-yl)-5-methyl-4-(3-pyridyl)-imidazole trihydrochloride (33) A mixture of **32** (1.0 g, 2.8 mmol), 10% Pd–C (1.0 g) and triethylamine (5 ml) in MeOH (105 ml) was hydrogenated at room temperature under atmospheric pressure. The reaction was monitored by TLC, and was completed within 5 h. After filtration, the filtrate was evaporated *in vacuo* and poured into a mixture of water and $CHCl_3$. The separated organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The resulting residue was dissolved with EtOH, and then a solution of HCl in MeOH was added. After evaporation, the resulting residue was recrystallized from EtOH–THF to give **33** (0.69 g, 62.7%). Analytical data of **33** are summarized in Table IV.

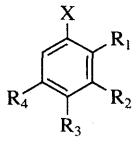
2-[4-Chloro-2-(2-ethoxycarbonyl)ethyl-7-methoxybenzimidazol-6-yl]-5-methyl-4-(3-pyridyl)imidazole (34) A mixture of 2-(4-acetylamino-5-

TABLE IV. Yield, Melting Point, and Analytical Data of 5-Methyl-4-(3-pyridinyl)-2-(substituted benzo[*d*]benzimidazol-5-yl)imidazole Derivaties

	Yield[a] (%)	Route[b]	mp (°C) (Recrystn. solvent)	Formula	Analysis (%) Calcd (Found)		
					C	H	N
23	43.4[c]	A	182—184 (EtOH–H_2O)	$C_{18}H_{17}N_5O \cdot 1H_2O$	64.08 (64.06	5.68 5.77	20.76 20.80)
24	45.6	B	158—163 (EtOH–H_2O)	$C_{19}H_{19}N_5O \cdot 2H_2O$	61.78 (61.87	6.28 6.05	18.96 18.65)
25	20.4	B	211—212 (EtOH–H_2O)	$C_{19}H_{18}N_6O_3 \cdot 5/2H_2O$	53.90 (54.06	5.46 5.50	19.85 19.53)
26	46.5[c]	B	214—215 (EtOH–H_2O)	$C_{18}H_{16}ClN_5O \cdot 5/4H_2O$	57.45 (57.51	4.96 4.97	18.61 18.50)
27	44.3[c]	A	>280 (AcOEt–MeOH)	$C_{17}H_{15}N_5 \cdot 4/5H_2O$	67.22 (67.39	5.51 5.56	23.06 23.17)
28	86.5[c]	A	235—241 (EtOH–H_2O)	$C_{18}H_{17}N_5 \cdot 4H_2O$	57.60 (57.64	6.71 6.34	18.66 18.20)
29	29.7	—	249—251 (EtOH–H_2O)	$C_{17}H_{15}N_5O_2 \cdot 1H_2O$	60.18 (60.51	5.07 5.45	20.63 20.72)
30	43.5[c]	—	>250 (EtOH)	$C_{17}H_{14}ClN_5O_2 \cdot 1/5H_2O$	56.82 (56.79	4.04 4.25	19.49 19.62)
31	12.0[d]	—	189—194 ($CHCl_3$–MeOH–Et_2O)	$C_{17}H_{14}ClN_5 \cdot 9/5H_2O$	57.33 (57.32	4.98 4.93	19.46 19.14)
32	27.9	D	245 (dec.) (AcOEt–MeOH)	$C_{18}H_{17}N_5O \cdot 2HCl \cdot 3H_2O$	48.44 (48.30	5.46 5.07	15.69 15.60)
33	62.7	—	>290 (EtOH–THF)	$C_{18}H_{16}ClN_5O \cdot 4/5H_2O$	60.49 (60.55	4.63 4.57	19.59 19.38)
34	73.2	—	184—186 (AcOEt–Et_2O)	$C_{22}H_{22}ClN_5O_3 \cdot 5/4H_2O$	57.15 (57.05	5.34 5.46	15.15 14.95)
35	45.0	—	230—233 (EtOH–Aceton)	$C_{20}H_{18}ClN_5O_3 \cdot 3HCl \cdot 9/5H_2O$	43.39 (43.46	4.48 4.54	12.65 12.51)
36	36.0	—	225—230 ($CHCl_3$–MeOH)	$C_{19}H_{19}N_5O \cdot 5/4H_2O$	64.12 (64.08	6.09 5.99	19.68 19.43)
37	69.0[b]	—	208—210 (EtOH–Et_2O)	$C_{18}H_{17}N_5O_2 \cdot 6/5H_2O$	60.57 (60.70	5.48 5.29	19.62 19.31)

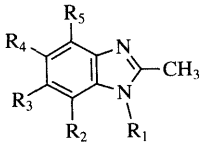
a) Yields of last reactions. *b*) A and B, see Chart 2; D, see Chart 4. *c*) Yield from **8**. *d*) Yield from **11**.

TABLE V. Physical Data of Methyl Benzoates and Benzaldehydes



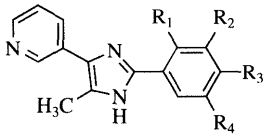
	X	R_1	R_2	R_3	R_4	Yield (%)	^{1}H-NMR (δ in DMSO-d_6)
5a	CHO	OCH_3	H	NHAc	NO_2	32.9	2.20 (3H, s), 4.07 (3H, s), 7.99 (1H, s), 8.38 (1H, s), 10.25 (1H, s, CHO), 10.65 (1H, br s)
5b	CHO	H	H	NHAc	NO_2	50.2	2.20 (3H, s), 7.80 (1H, d, J=9 Hz), 8.20 (1H, d, J=9 Hz), 8.44 (1H, s), 10.23 (1H, s)
5c	CHO	CH_3	H	NHAc	NO_2	50.0	2.20 (3H, s), 2.72 (3H, s), 7.78 (1H, s), 8.45 (1H, s), 10.20 (1H, s), 10.50 (1H, br s)
5d	CHO	OCH_3	Cl	NH_2	NO_2	38.9	4.00 (3H, s), 8.00 (2H, br s), 8.48 (1H, s), 10.00 (1H, s)
14	$COOCH_3$	OCH_3	NO_2	NHAc	Cl	89.3	2.05 (3H, s), 3.95 (6H, s), 8.20 (1H, s), 10.35 (1H, br s)
15	CHO	OCH_3	NO_2	NHAc	Cl	84.0	2.10 (3H, s), 4.00 (3H, s), 8.18 (1H, s), 10.19 (1H, s), 10.40 (1H, s)
19	$COOCH_3$	OCH_3	H	$N(CH_3)Ac$	NO_2	87.8	1.85, 2.21 (3H, both s), 3.15, 3.55 (3H, both s), 3.90 (3H, s), 4.05 (3H, s), 7.30, 7.55 (1H, both s), 8.30, 8.50 (1H, both s)
20	CHO	OCH_3	H	$N(CH_3)Ac$	NO_2	88.8	1.78, 2.15 (3H, both s), 3.10, 3.48 (3H, both s), 4.08 (3H, s), 7.40, 7.58 (1H, both s), 8.15, 8.38 (1H, both s), 10.30 (1H, s, CHO)

TABLE VI. Physical Data of Benzimidazoles



	R_1	R_2	R_3	R_4	R_5	Yield (%)	mp (°C)	^{1}H-NMR (δ in DMSO-d_6)
12	H	H	Cl	$COOCH_3$	H	99.5	114—118	2.55 (3H, s), 3.95 (3H, s), 8.00 (1H, s), 8.30 (1H, s)
13	H	H	Cl	CHO	H	57.6	180—184	2.50 (3H, s), 7.95 (1H, s), 8.22 (1H, s), 10.40 (1H, s, CHO)
17	H	OCH_3	$COOCH_3$	H	Cl	86.9	188—191	2.55 (3H, s), 3.90 (3H, s), 3.95 (3H, s), 7.60 (1H, s)
18	H	OCH_3	CHO	H	Cl	33.9	239—240	2.53 (3H, s), 3.98 (3H, s), 7.63 (1H, s), 10.35 (1H, s)
21	CH_3	H	OCH_3	CHO	H	63.4	162—165	2.50 (3H, s), 3.68 (3H, s), 3.94 (3H, s), 7.12 (1H, s), 7.75 (1H, s), 10.30 (1H, s)

TABLE VII. Physical Data of 5-Methyl-4-(3-pyridyl)-2-(substituted phenyl)imidazoles



	R_1	R_2	R_3	R_4	Yield (%)	mp (°C)	^{1}H-NMR (δ in DMSO-d_6, J=Hz)
8a	OCH_3	H	NHAc	NO_2	78.5	193—197	2.20 (3H, s, Ac), 2.52 (3H, s, CH_3), 4.05 (3H, s, OCH_3), 7.45 (1H, dd, J=8, 5, 5′-py), 7.90 (1H, s, ph), 8.10 (1H, ddd, J=8, 2, 2, 4′-py), 8.49 (1H, dd, J=5, 2, 6′-py), 8.80 (1H, s, ph), 8.95 (1H, d, J=2, 2′-py), 10.40 (1H, s, NH)
8b	H	H	NHAc	NO_2	55.2	260—265	2.13 (3H, s), 2.51 (3H, s), 7.47 (1H, dd, J=8, 5), 7.83 (1H, d, J=9), 8.00—8.63 (3H, m), 8.55 (1H, d, J=2), 8.95 (1H, d, J=2), 10.37 (1H, br s)
8c	CH_3	H	NHAc	NO_2	52.6	234—238	2.16 (3H, s), 2.52 (3H, s), 2.70 (3H, s), 7.45 (1H, dd, J=8, 5), 7.70 (1H, s), 8.10 (1H, ddd, J=8, 2, 2), 8.50 (1H, s), 8.67 (1H, dd, J=5, 2), 9.0 (1H, d, J=2), 10.33 (1H, s)
8d	OCH_3	Cl	NH_2	NO_2	49.2	216—222	2.50 (3H, s), 3.90 (3H, s), 7.3—7.8 (2H, m), 3.08 (1H, d, J=8), 8.43 (1H, d, J=5), 8.90 (1H, s)
10	OCH_3	H	NH_2	NH_2	84.7	*a)*	
11	OCH_3	Cl	NH_2	NH_2	*b)*		
16	OCH_3	NO_2	NHAc	Cl	59.9	190—193	2.05 (3H, s), 2.52 (3H, s), 3.80 (3H, s), 7.40 (1H, dd, J=8, 4), 8.07 (1H, d, J=8), 8.43 (1H, m), 8.92 (1H, br s), 10.77 (1H, s), 12.55 (1H, br s)
22	OCH_3	H	$N(CH_3)Ac$	NO_2	*b)*		

a) Physical data are described in the experimental section. *b)* These compounds were used for the following reaction without isolation.

chloro-2-methoxy-3-nitrophenyl)-5-methyl-4-(3-pyridyl)imidazole (**16**, 3.00 g, 7.48 mmol) in 6 N HCl (40 ml) was heated at 85—90 °C for 9 h. The reaction mixture was poured into water (100 ml), and adjusted to pH 7 with 20% K_2CO_3. After extraction with AcOEt, the organic layer was dried over $MgSO_4$. After filtration, the filtrate was evaporated *in vacuo*. The resulting precipitate was washed with IPE and dried to give 2-(4-amino-5-chloro-2-methoxy-3-nitrophenyl)-5-methyl-4-(3-pyridyl)-imidazole (2.20 g, 63.2%). The nitro derivative was added to a refluxing mixture of Fe (1.70 g, 28.3 mmol), NH_4Cl (0.16 g, 3 mmol) in EtOH (40 ml) and water (4 ml). The reaction mixture was stirred and refluxed for 2 h. After removal of the insoluble material, the filtrate was evaporated *in vacuo*. The resulting residue was dissolved with a mixture of $CHCl_3$ and MeOH and the solution was dried over $MgSO_4$. After filtration, the filtrate was evaporated *in vacuo* to give 2-(3,4-diamino-5-chloro-2-methoxyphenyl)-5-methyl-4-(3-pyridyl)imidazole (1.60 g, 100.8%), which was used for the next reaction without further purification.

An ice-cooled mixture of the above diaminophenyl derivative (1.5 g, 4.5 mmol) and trimethylsilyl *N*-(trimethylsilyl)acetimidate (3 ml) in THF (50 ml) was treated with ethyl 3-chlorocarbonylpropionate (1.5 g, 9 mmol), and the whole mixture was stirred at room temperature for 3.5 h. After concentration *in vacuo*, EtOH (50 ml) and sulfonic acid (3 ml) were added to the resulting residue. The whole mixture was stirred and refluxed for 30 min, then evaporated *in vacuo*. The resulting residue was taken up in water and AcOEt. The separated water layer was adjusted to pH 8 with 20% K_2CO_3 and extracted with AcOEt. The solution was dried over $MgSO_4$ and evaporated *in vacuo*, and the resulting precipitate was recrystallized from AcOEt and EtOH to give **34** (1.45 g, 73.2%). IR (Nujol): 3400, 3225, 1740, 1660, 1540 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.20 (3H, t, J=8 Hz, $CH_2C\underline{H}_3$), 2.57 (3H, s, CH_3), 2.67 (4H, br s, CH_2CH_2), 3.75 (3H, s, OCH_3), 4.17 (2H, q, J=8 Hz, $C\underline{H}_2CH_3$), 7.40 (1H, dd, J=8, 5 Hz), 8.07 (1H, s), 3.20 (1H, ddd, J=8, 2, 2 Hz), 8.42 (1H, dd, J=5, 2 Hz), 8.90 (1H, d, J=2 Hz). Other analytical data of **34** are given in Table IV.

2-[2-(2-Carboxy)ethyl-4-chloro-7-methoxybenzimidazol-6-yl]-5-methyl-4-(3-pyridyl)imidazole Trihydrochloride (35) A mixture of **34** (0.85 g, 1.89 mmol) and 6 N HCl (10 ml) was stirred at 80 °C for 4.5 h. After evaporation *in vacuo*, the resulting precipitate was recrystallized from EtOH and acetone to give **35** (0.45 g, 45.0%). IR (Nujol): 3350, 1720, 1610, 1565 cm^{-1}. ^{1}H-NMR (D_2O) δ: 2.73 (3H, s, CH_3), 3.20 (2H, t, J=6 Hz, CH_2CH_2), 3.57 (2H, t, J=6 Hz, CH_2CH_2), 4.25 (3H, s, OCH_3), 7.93 (1H, s), 8.39 (1H, dd, J=8, 5 Hz), 8.85—9.13 (2H, m), 9.28 (1H, d, J=2 Hz). Other analytical data of **35** are given in Table IV.

Pharmacological Tests Platelet Aggregation Studies[8] *Ex Vivo* Studies: Male Sprague-Dawley rats weighing about 250 g were used after overnight fasting. One hour after oral administration of the test compound (32 mg/kg or 10 mg/kg) or vehicle (control), blood was collected into a tube containing 0.1 vol. of 3.8% sodium citrate. To 0.45 ml of blood, 0.05 ml of collagen (final concentration 5.0 (μg/ml) was added, and the mixture was incubated for 5 min at 37 °C under shaking. The reaction was terminated by addition of 1 ml of 10 mM phosphate-buffered saline (pH 7.4) containing 11.5 mM *N,N,N',N'*-tetra(carboxylmethyl)-1,2-diaminoethane and 1% formalin. The reaction mixture was centrifuged at 70 × *g* for 5 min and the platelet count of the upper phase was measured with a Platelet Analyzer 810 (Backer Instruments). Platelet aggregation was calculated according to the following formula:

$$\text{platelet aggregation } (\%) = (A-B)/A \times 100$$

A: platelet count after addition of vehicle
B: platelet count after addition of collagen

$$\text{inhibition } (\%) = (C-D)/C \times 100$$

C: platelet aggregation (%) of control
D: platelet aggregation (%) of test compound

In Vitro Studies: Male Japanese White rabbits weighting about 2 kg were used. Blood was collected into plastic vessels containing 3.8% sodium citrate (1 volume with 9 volumes of blood). Platelet-rich plasma (PRP) was obtained by centrifugation of the blood at 120 × *g* for 15 min. To 225 μl of PRP, 25 μl of test compound dissolved in 25 mM Tris–acetate solution (pH = 7.4) containing 120 mM NaCl was added, and the mixture was stirred for 2 min at 37 °C. To this solution, 5 μl of collagen (125 μg/ml) was added to induce aggregation. Aggregation was measured using an aggregometer (NKK Hema-Tracer 1) and calculated according to the above formula. Activities of inhibitors (test compounds) were expressed as IC_{50} values, *i.e.*, doses required to inhibit the platelet aggregation response by 50%.

TXA_2 Synthetase-Inhibitory Activity[9] Aspirin-treated human platelet microsomes (APM, Ran Biochem, Israel) were used as a source of TXA_2 synthetase. APM was suspended in 50 mM Tris–HCl buffer (pH 7.5) containing 0.1 M NaCl. To 90 μl of APM suspension, 10 μl of test drug solution was added and the mixture was preincubated for 3 min at 25 °C. To this reaction mixture, 2 μl of PGH_2 solution (10 μg/ml in acetone) was added and the entire mixture was incubated for 3 min at 25 °C. The reaction was stopped by the addition of 10 μl of $FeCl_2$ solution (25 mM in H_2O) and the mixture was left for 15 min at room temperature. The reaction mixture was centrifuged at 10000 rpm for 5 min.

TXB_2 in the supernatant was measured by radioimmunoassay (Amersham). IC_{50} (concentration inhibiting TXB_2 production by 50%) values were graphically calculated.

Cyclooxygenase-Inhibitory Activity[10] Microsomal fraction from sheep seminal vesicles (Ran Biochem) was used as a source of cyclooxygenase. The reaction mixture consisted of 0.1 M Tris–HCl (pH 7.6), 1 mM epinephrine, 2 mM glutathione, 240 μg of the microsomes, and the drug to be tested. The reaction was started by the addition of 0.1 mM [^{14}C]arachidonic acid (58 mCi/mmol), then the mixture was incubated at 37 °C for 5 min and the reaction was stopped by the addition of 50 μl of 1 N HCl. Prostaglandins were extracted with 1.5 ml of AcOEt, and the separated organic layer was dried with nitrogen gas, dissolved in 40 μl of MeOH and applied to a thin-layer plate (Merck, Kieselgel 60F). The solvent used for the chromatography was a mixture of AcOEt and acetic acid (100 : 2). The PGE_2 fraction was scraped off and the radioactivity was counted in a toluene scintillator.

Phosphodiesterase-Inhibitory Activity[11] Cyclic AMP phosphodiesterase was obtained from rabbit platelets. PRP was centrifuged at 1000 × *g* for 10 min and the pellet was suspended in 25 mM Tris–acetate buffer (pH 7.4) containing 120 mM NaCl. The pellet was washed twice using the same buffer and finally resuspended in 40 mM Tris–HCl buffer (pH 7.4). The suspension of cells was sonicated 3 times for 10 s (Tomy, UR-150P). The platelet lysate was centrifuged at 10000 × *g* for 20 min and then recentrifuged at 100000 × *g* for 60 min. The supernatant was stored at −70 °C and used as phosphodiesterase.

The phosphodiesterase activity was measured in 500 μl of reaction mixture consisting of 40 mM Tris–HCl buffer (pH 7.4), 1 mM $MgCl_2$, 0.15 μM cyclic AMP (containing 10 nM ^{3}H-cyclic AMP) and the crude cytosolic enzyme (approximately 10 μg protein). After 10 min at 30 °C, the reaction was terminated by immersing the reaction tube in a boiling water bath for 2.5 min. Snake venom (50 μl of 1 mg/ml *Atrox crotalus*) was then added for 10 min at 30 °C to convert the 5′-AMP to the uncharged nucleotide, adenosine. An ion-exchange resins slurry (AG 1X2, 1 ml) was added to bind all of the unconverted cyclic AMP. After centrifugation, an aliquot (0.25 ml) of the supernatant was removed for quantitative analysis in a liquid scintillation counter.

Vasorelaxant Activity Helical strips of rat thoracic aorta were suspended in an organ bath containing Tyrode solution gassed with 95% O_2–5% CO_2 at 37 °C under 0.5 g load. Contraction was induced by addition of KCl solution (final concentration, 30 mM). After the tonus had reached a plateau, drug solution (dissolved in dimethylsulfoxide) was added cumulatively and finally 0.1 mM papaverine was added to obtain maximum relaxation. Activities of the test compound were expressed as ED_{50} values, *i.e.*, dose required to relax the isolated rat aorta by 50%.

References and Notes

1) Part I: A. Tanaka, K. Ito, S. Nishino, Y. Motoyama, H. Takasugi, *Chem. Pharm. Bull.*, **40**, 3206 (1992).
2) H. Takasugi, K. Itoh, S. Nishino, A. Tanaka, T. Takaya, Japan. Patent 01 258 670 (1989).
3) Steering Committee of the Physicians' Health Study Research Group, *N. Engl. J. Med.*, **321**, 129 (1989); Stroke Prevention in Atrial Fibrillation Investigators, *Circulation*, **84**, 527 (1991); M. Gent, J. A. Blakely, J. D. Easton, D. J. Ellis, V. C. Hachinski, J. W. Harbison, E. Panak, R. S. Roberts, J. Scicuirella, A. G. G. Turpie, *Lancet*, i, 1215 (1989).
4) L. A. Harker, *Semin. Thromb. Hemostasis*, **12**, 134 (1986); L. A. Harker, V. Fuster, *J. Am. Coll. Cardiol.*, **8**, 21B (1986).
5) C. Patrono, *Trends in Pharm. Sci.*, **10**, 453 (1989).
6) P. N. Preston, "Heterocyclic Compounds, Benzimidazoles and

Congeneric Tricyclic Compounds," Vol. 40, ed. by A. Weissberger, E. C. Tayler, John Wiley & Sons, Inc., New York, 1981; S. Ram, D. S. Wise, L. L. Wotring, J. W. McCall, L. B. Townsend, *J. Med. Chem.*, **35**, 539 (1992).
7) H. J. Lucas, E. R. Kennedy, "Organic Syntheses," Coll. Vol. III, ed. by E. C. Horning, John Wiley & Sons, Inc., New York, 1955, p. 482.
8) G. C. R. Born, M. J. Cross, *J. Physiol.*, **168**, 178 (1963).
9) B. Hammarston, P. Faladaeu, *Proc. Natl. Acad. Sci. U.S.A.*, **74**, 3691 (1977).
10) K. Matsuda, K. Ohnishi, E. Misaka, M. Yamazaki, *Biochem. Pharmacol.*, **32**, 1347 (1983).
11) G. Brooher, L. J. Thomas, Jr., M. M. Appleman, *Biochemistry*, **7**, 4177 (1968).
12) For simplicity of discussion, the numbering of the benzimidazole ring in this manuscript follows the IUPAC numbering: