Synthesis and Platelet Aggregation Inhibitory Activity of Diphenylazole Derivatives. I. Thiazole and Imidazole Derivatives

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Diphenylimidazole and diphenylthiazole derivatives were synthesized and tested as inhibitors of platelet aggregation in *in vitro* experiments with the rabbit. Diphenylthiazole derivatives (10) were more potent than diphenylimidazole derivatives (4) in inhibiting arachidonic acid-induced platelet aggregation of rabbit platelet-rich plasma. Two diphenylimidazole and eight diphenylthiazole derivatives were evaluated for *ex vivo* arachidonic acid and collagen-induced platelet aggregation inhibitory activity using guinea pigs. In these compounds, 4,5-bis(4-methoxyphenyl)-2-(1,5-dimethyl-2-pyrrolyl)thiazole (10n) showed strong activity *in vitro* and *ex vivo*. The *ex vivo* activity of 10n was 200 times stronger than that of aspirin. The mechanism of the activity of 10n was the inhibition of cyclo-oxygenase.

Keywords platelet aggregation inhibitor; diphenylthiazole; diphenylazole; diphenylimidazole; anti platelet activity

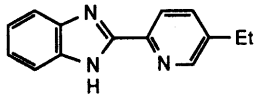
In recent years, many compounds possessing platelet aggregation inhibitory activity have been discovered. The mechanisms of inhibition vary. For example, cyclo-oxygenase inhibitor (aspirin[1]), thromboxane synthetase inhibitor (ozagrel[2]), adenylate cyclase activator (ticlopidine[3]), phosphodiesterase inhibitor (dipyridamole[4] and cilostazole[5]) are known inhibitors and have been investigated for application to ischemic disease. Among these compounds, aspirin has been studied most extensively.

One of the causes of ischemic disease is platelet aggregation. Thromboxane A_2 is derived from arachidonic acid released from phospholipids and induces platelet aggregation. Another cause of ischemic disease is vasospasms, which can be induced by thromboxane A_2. Therefore, use of drugs that inhibit platelet function and suppress the synthesis of thromboxane A_2 should be effective in preventing and curing ischemic disease. The platelet aggregation inhibitory activity of aspirin is due to a decrease in thromboxane A_2 by the inhibition of cyclo-oxygenase[6] in platelets. In double blind trials, aspirin improved symptoms of ischemic disease. Aspirin was effective in myocardial infarction,[7–9] unstable angina,[10,11] and transition ischemic attack.[12–15] However, the platelet aggregation inhibitory activity of aspirin was weak. Moreover, aspirin has been known to induce stomach ulcers.[16,17] The stomach ulcerogenic activity of aspirin is based on a decrease in prostaglandin I_2 and prostaglandin E_1 due to the inhibition of cyclo-oxygenase. Prostaglandin I_2 and prostaglandin E_1 maintain stomach mucosa membrane blood flow and protect stomach cells.[18] We reported that a nonacidic 2-heteroarylbenzimidazole derivative, KB-1043, possessed anti-inflammatory activity.[19] Although one of the mechanisms of the activity of KB-1043 was the inhibition of cyclo-oxygenase, the ulcerogenic activity of KB-1043 was weak. We expected that a nonacidic cyclo-oxygenase inhibitor would eliminate the ulcerogenic activity. On the other hand, the idea of using a phenyl group or two phenyl groups attached to a ring system to mimic the corresponding benzo analog (fused phenyl moiety) has often been explored in medicinal chemistry.[20]

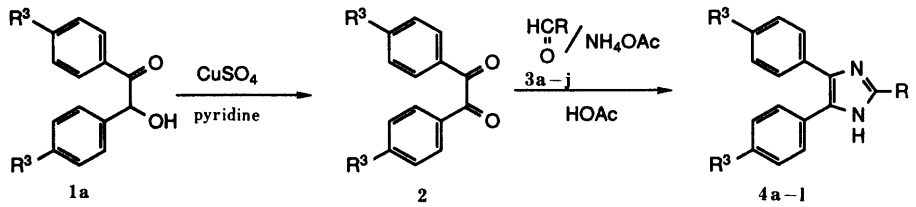
In a continuous investigation of 2-heteroarylazole derivatives, we synthesized a variety of 2-heteroaryl-4,5-diphenylazole and 2-heteroaryl-4-phenylazole derivatives as 2-heteroarylbenzimidazole derivative analog and screened them for platelet aggregation inhibitory activity.

Chemistry 4,5-Diphenylimidazole derivatives (**4a, f**) were prepared according to the literature.[21] 4,5-Bis(4-methoxyphenyl)imidazole derivatives (**4b, d, g—l**) were prepared by condensation of anisil (**2**) with aldehydes (**3**) and ammonium acetate in acetic acid as shown in Chart 1. In the case of **4c** and **4e**, because aldehydes were acid labile, dimethyl sulfoxide (DMSO) was used as a solvent instead of acetic acid. Anisil (**2**) was prepared by oxidation of anisoin (**1a**) with cupric sulfate in pyridine according to the literature.[22]

Diphenylthiazole derivatives (**10**) were synthesized by condensation of bromodeoxybenzoin derivatives (**9**)[23,24] and thioamides (**7**)[25–32] in acetonitrile as shown in Chart 3. Pyrrolecarbothioamides (**7k, l**) were prepared from pyrrolecarbaldehydes (**3k, l**) through 3-step reactions as shown in Chart 2. Pyrrolecarbaldehydes (**3k, l**) were condensed with hydroxylamine to give oximes (**5k, l**).[33] The oximes (**5k, l**) were dehydrated with acetic anhydride to give nitriles



KB-1043



1a 2 4a–l

Chart 1

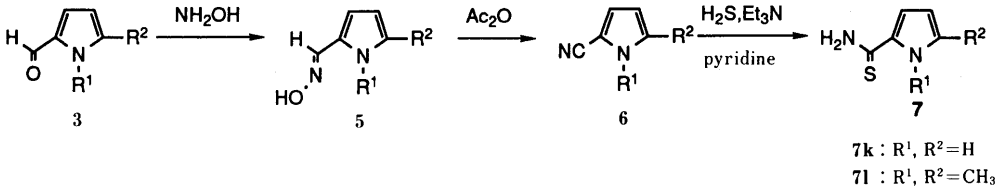


Chart 2

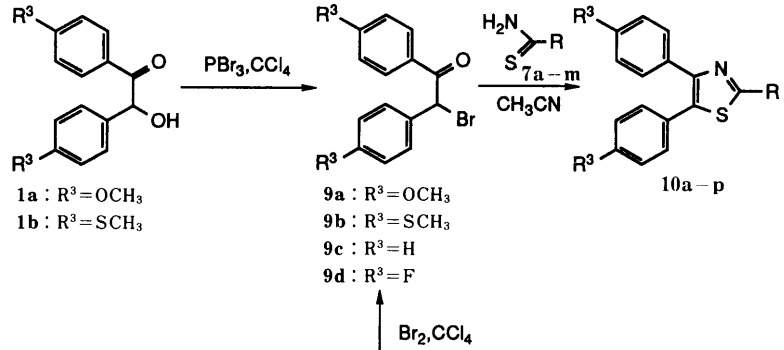


Chart 3

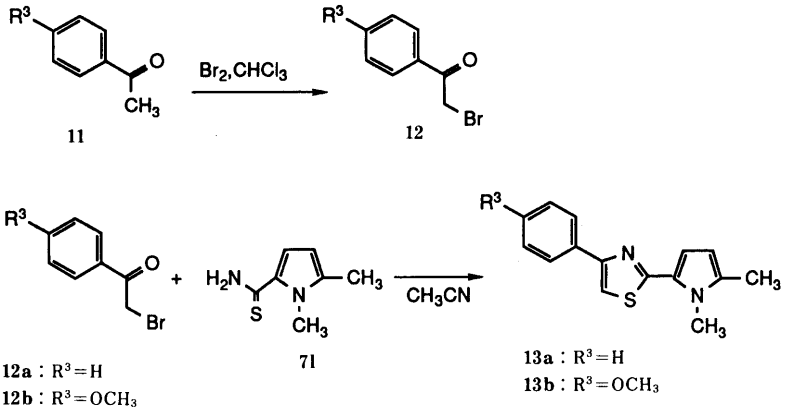


Chart 4

(**6k**, **l**).[34] The nitriles (**6k**, **l**) were treated with hydrogen sulfide in pyridine in the presence of triethylamine to give pyrrolecarbothioamides (**7k**, **l**).

Bromodeoxybenzoins (**9**) were prepared by two methods. Bromodeoxyanisoin (**9a**) and α-bromo-4,4′-dimethylthiodeoxybenzoin (**9b**) were obtained by the reaction of benzoin derivatives (**1a**, **b**) and phosphorous tribromide in carbon tetrachloride.[23] Bromodeoxybenzoin (**9c**) and α-bromo-4,4′-difluorodeoxybenzoin (**9d**) were obtained by bromination of deoxybenzoin derivatives (**8c**, **d**), in carbon tetrachloride.[24] These deoxybenzoin derivatives (**8c**, **d**) were easily obtained by the reduction of benzoin derivatives (**1c**, **d**). 4,4′-Difluorodeoxybenzoin (**8d**) was prepared by the reduction of 4,4′-difluorobenzoin (**1d**) with tin, hydrochloric acid, and cupric sulfate in ethanol.[34]

Phenylthiazole derivatives (**13**) were prepared by the condensation of phenacyl bromide derivatives (**12**) with pyrrolecarbothioamide derivative (**7l**) in acetonitrile as shown in Chart 4. Phenacyl bromide derivatives (**12**) were obtained by bromination of acetophenone derivatives (**11**).[35] The syntheses of these compounds are summarized in Tables I through VI.

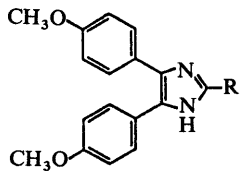
Results and Discussion

The diphenylazole and phenylthiazole derivatives were evaluated for *in vitro* arachidonic acid-induced platelet aggregation inhibitory activity using rabbit platelet-rich plasma (PRP). Results are given in Tables VII and VIII.

Almost all of the examined diphenylazole derivatives exhibited excellent platelet aggregation inhibitory activity. Diphenylthiazole derivatives generally showed stronger inhibitory activity on the aggregation than the corresponding diphenylimidazole derivatives. Structure–activity relationships were different between the diphenylthiazole series and diphenylimidazole series.

Structure–Activity Relationships of Diphenylimidazole Series The substituent effects on the 4-position of the benzene rings and the 2-position of the imidazole ring were investigated and results are shown in Table VII.

TABLE I. Synthesis of 4,5-Bis(4-methoxyphenyl)imidazole Derivatives (**4**)



	R	Solvent	Yield (%)	Isolation[a]	mp (°C)
4b	3-Pyridyl·H_2O	AcOH	78	Benzene	188.0—190.5
4c	2-Pyridyl	DMSO	48	Benzene	190.0—191.0
4d[b]	6-Methyl-2-pyridyl	AcOH	10	Benzene	183.5—184.5
4e	4-Pyridyl	DMSO	33	CH_3CN	182.0—185.0
4g	2-Thienyl	AcOH	46	Benzene	195.0—196.0
4h	5-Methyl-2-thienyl	AcOH	19	Benzene	186.0—187.0
4i	3-Methyl-2-thienyl	AcOH	86	Benzene	185.5—186.5
4j	3-Thienyl	AcOH	64	Benzene	201.5—204.0
4k	2-Pyrrolyl	AcOH	37	Benzene	229.5—231.0
4l	1,5-Dimethyl-2-pyrrolyl	AcOH	30	Benzene	173.5—174.5

a) Recrystallization solvent. *b*) Diethyl acetal was used instead of aldehyde.

4,5-Bis(4-methoxyphenyl) derivatives (**4b, g**) showed stronger activity than diphenyl derivatives (**4a, f**). These results showed that the introduction of methoxy groups at the 4-position of the benzene rings enhanced the activity.

The activity of 4,5-bis(4-methoxyphenyl) derivatives were affected by a heteroaryl substituent at the 2-position of the imidazole ring. The 2-pyrrolyl group (**4k**) was the most active substituent. 3-Pyridyl (**4b**), 2-pyridyl (**4c**), and 2-thienyl (**4g**) derivatives were 2-fold less active than the 2-pyrrolyl derivative (**4k**). Substitution with the 3-thienyl group (**4j**) at the 2-position of the imidazole ring led to an 8-fold drop in the activity when compared with **4k**. The 4-pyridyl derivative (**4e**) exhibited only weak activity. Introduction of methyl groups at the 1- and 5-positions of the pyrrole ring (**4 l**) decreased the activity. In the case of the thiophene ring, the substituent effect of a methyl group was not uniform. Introduction of a methyl group at the 5-position of the thiophene ring (**4h**) drastically decreased the activity. On the other hand, 3-methyl-2-thienyl derivative (**4i**) increased the activity 2-fold when compared with the 2-thienyl derivative (**4g**).

In the diphenylimidazole series, **4i** and **4k** exhibited strong activity. The 50% inhibitory concentration (IC_{50}) values of these derivatives were in 10^{-8} M order.

Structure–Activity Relationships of Diphenylthiazole Series The substituent effects on the 4-position of the benzene rings and the 2-position of the thiazole ring were investigated and the results are shown in Table VII.

Introduction of the methoxy groups at the 4-position of the benzene rings (**10n**) increased 500-fold platelet aggregation inhibitory activity compared with diphenyl derivative (**10 l**). Substitution of the methoxy groups at the 4-position of the benzene rings with fluorine atoms (**10m**) decreased

TABLE II. Elemental Analysis of 4,5-Bis(4-methoxyphenyl)imidazole Derivatives (**4**)

	R	Formula	Analysis (%) Calcd			Found		
			C	H	N	C	H	N
4b	3-Pyridyl·H_2O	$C_{22}H_{21}N_3O_3$	70.38	5.60	11.19	69.98	5.61	11.24
4d	6-Methyl-2-pyridyl	$C_{23}H_{21}N_3O_2$	74.37	5.70	11.31	74.50	5.61	11.29
4e	4-Pyridyl	$C_{22}H_{19}N_3O_2$	73.93	5.36	11.76	74.19	5.26	11.86
4g	2-Thienyl	$C_{21}H_{18}N_2O_2S$	69.50	4.96	7.73	69.60	5.10	7.60
4h	5-Methyl-2-thienyl	$C_{22}H_{20}N_2O_2S$	70.19	5.35	7.44	70.17	5.37	7.39
4i	3-Methyl-2-thienyl	$C_{22}H_{20}N_2O_2S$	70.19	5.35	7.44	70.36	5.20	7.51
4j	3-Thienyl	$C_{21}H_{18}N_2O_2S$	69.50	4.96	7.73	69.24	5.18	7.69
4l	1,5-Dimethyl-2-pyrrolyl	$C_{23}H_{23}N_3O_2$	73.97	6.21	11.25	73.80	6.06	11.19

TABLE III. ^{1}H-NMR Spectra of 4,5-Bis(4-methoxyphenyl)imidazole Derivatives (**4**)

	R	^{1}H-NMR chemical shifts[a] (δ)
4b	3-Pyridyl·H_2O	3.7 (6H, s), 6.6—6.85 (4H, m), 6.9—7.4, (5H, m+1H, br)
4d	6-Methyl-2-pyridyl	2.4 (3H, s), 3.7 (6H, s), 6.4—8.1 (11H, m+1H, br)
4e	4-Pyridyl	3.8 (6H, s), 6.8—7.15 (2H, m), 7.35—7.6, (4H, m), 7.9—8.05 (2H, m), 8.55—8.7 (2H, m), 12.4—13.0 (1H, br)
4g	2-Thienyl	3.7 (6H, s), 6.45—6.8 (6H, m), 6.95—7.4 (5H, m), 8.0—8.4 (1H, br)
4h	5-Methyl-2-thienyl	2.45 (3H, s), 3.7 (6H, s), 6.55—6.85 (4H, m), 7.0—7.45 (6H, m), 8.9—9.4 (1H, br)
4i	3-Methyl-2-thienyl	2.5 (3H, s), 3.7 (6H, s), 6.5—6.85 (4H, m), 7.0—7.45 (6H, m), 8.4—8.9 (1H, br)
4j	3-Thienyl	3.7 (6H, s), 6.45—6.8 (4H, m), 7.0—7.55 (7H, m), 8.1—8.45 (1H, br)
4l	1,5-Dimethyl-2-pyrrolyl	2.15 (3H, s), 3.8 (6H, s), 4.0 (3H, s), 5.95 (1H, d, J=2), 6.3 (1H, d, J=2), 6.8—6.95 (4H, m), 7.3—7.55 (4H, m), 8.4—9.5 (1H, br)

a) Measured in $CDCl_3$.

the activity. Substitution of oxygen atoms at the methoxy groups of **10n** with sulfur atoms (**10o**) also decreased the activity. These results showed that the methoxy groups at the 4-position of the benzene rings are very important for platelet aggregation inhibitory activity.

In the 4,5-bis(4-methoxyphenyl) derivatives, 2-pyrrolyl (**10k**), 2-thienyl (**10f**), 3-thienyl (**10j**), and 2-pyridyl (**10b**) derivatives exhibited nearly equal inhibitory activity. The IC_{50} values of these derivatives were about 1×10^{-7} M. The 3-pyridyl derivative (**10a**) showed only weak activity. Pyrazinyl derivative (**10p**), possessing 2 nitrogen atoms in the 6-membered heteroaromatic ring, was 2-fold less active than the 2-pyridyl derivative (**10b**), possessing 1 nitrogen atom in the 6-membered heteroaromatic ring. The effects of introduction of a methyl, a chloro, and a methoxy group into the heteroaryl rings were examined. In the case of the pyrrole ring (**10n**), introduction of methyl groups enhanced the activity. The activity of (1,5-dimethyl-2-pyrrolyl) derivative (**10n**) was fairly strong compared with the unsubstituted pyrrolyl derivative (**10k**). On the other hand, in the thiophene or the pyridine ring, introduction of a methyl group reduced (**10i**) or had no effect (**10c, d, g**) on the activity. With introduction of a chlorine atom into the thienyl derivative (**10h**), the activity was nearly equal to the 2-thienyl derivative (**10f**). Introduction of a methoxy group into the 2-pyridyl derivative (**10e**) decreased the activity 4-fold when compared with the 2-pyridyl derivative (**10b**).

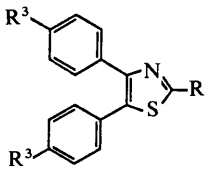
Among the diphenylthiazole derivatives, **10b—d, f—h, k,** and **10n** exhibited strong activity. The IC_{50} values of these derivatives were in 10^{-8} M order.

Platelet Aggregation Inhibitory Activity of 4-Substituted-Phenylthiazole Derivatives The substituent effect on the 5-position of the thiazole ring was investigated and results are shown in Table VIII.

4-Phenylthiazole derivatives (**13a, b**) did not exhibit any inhibitory activity. From these results, it seems that the 4,5-diphenylthiazole moiety is necessary for platelet aggregation inhibitory activity.

***Ex Vivo* Activity** Ten derivatives which showed strong activity (the IC_{50} values of these derivatives were in 10^{-8} M order) were evaluated for *ex vivo* arachidonic acid and collagen-induced platelet aggregation inhibitory activity using guinea pigs. Two compounds (**4i, k**) were diphenylimidazole derivatives. Eight other compounds (**10b—d, f—h, k, n**) were diphenylthiazole derivatives. The results are given in Table IX. All of the diphenylimidazole derivatives (**4i, k**) and all of the 2-unsubstituted-heteroaryl-diphenylthiazole derivatives (**10b, f, k**) showed no activity at a dose of 1 mg/kg. The introduction of substituents at the heteroaryl group, in some cases (**10d, h, n**), enhanced the activity. In these three compounds, 4,5-bis(4-methoxyphenyl)-2-(1,5-dimethylpyrrol-2-yl)thiazole (**10n**) showed the strongest *ex vivo* platelet aggregation inhibitory activity. The activity of **10n** in *ex vivo* was 200 times stronger than

TABLE IV. Synthesis of 4,5-Diphenylthiazole Derivatives (**10**)



	R	R^3	Yield (%)	Isolation[a]	mp (°C)
10a	3-Pyridyl	MeO	40	Ligroin	138.5—140.0
10b	2-Pyridyl	MeO	69	Ligroin	144.0—146.0
10c	6-Methyl-2-pyridyl	MeO	60	Ligroin	90.0— 92.0
10d	4-Methyl-2-pyridyl	MeO	62	Ligroin	136.0—137.5
10e	6-Methoxy-2-pyridyl	MeO	50	EtOH	124.0—125.0
10f	2-Thienyl	MeO	61	EtOH	139.5—140.5
10g	5-Methyl-2-thienyl	MeO	59	Ligroin	148.5—149.5
10h	5-Chloro-2-thienyl	MeO	39	Ligroin	157.0—158.0
10i	3-Methyl-2-thienyl	MeO	43	EtOH	95.5— 98.5
10j	3-Thienyl	MeO	50	EtOH	133.5—134.5
10k	2-Pyrrolyl	MeO	86	Ligroin	131.5—134.0
10l	1,5-Dimethyl-2-pyrrolyl	H	91	Hexane	119.0—120.0
10m	1,5-Dimethyl-2-pyrrolyl	F	38	Hexane	135.0—136.0
10n	1,5-Dimethyl-2-pyrrolyl	MeO	75	Cyclohexane	116.5—117.5
10o	1,5-Dimethyl-2-pyrrolyl	MeS	64	Cyclohexane	112.0—113.5
10p	Pyrazinyl	MeO	42	AcOEt	148.0—149.0

a) Recrystallization solvent.

TABLE V. Elemental Analysis of 4,5-Diphenylthiazole Derivatives (**10**)

	R	R^3	Formula	Analysis (%) Calcd			Found		
				C	H	N	C	H	N
10a	3-Pyridyl	MeO	$C_{22}H_{18}N_2O_2S$	70.57	4.84	7.48	70.74	4.66	7.51
10b	2-Pyridyl	MeO	$C_{22}H_{18}N_2O_2S$	70.57	4.84	7.48	70.80	4.66	7.47
10c	6-Methyl-2-pyridyl	MeO	$C_{23}H_{20}N_2O_2S$	71.11	5.19	7.21	71.41	5.08	7.13
10d	4-Methyl-2-pyridyl	MeO	$C_{23}H_{20}N_2O_2S$	71.11	5.19	7.21	71.45	5.05	7.14
10e	6-Methoxy-2-pyridyl	MeO	$C_{23}H_{20}N_2O_3S$	68.30	4.98	6.93	68.55	4.91	6.78
10f	2-Thienyl	MeO	$C_{21}H_{17}NO_2S_2$	66.47	4.52	3.69	66.34	4.30	3.74
10g	5-Methyl-2-thienyl	MeO	$C_{22}H_{19}NO_2S_2$	67.15	4.87	3.56	67.15	4.63	3.49
10h	5-Chloro-2-thienyl	MeO	$C_{21}H_{16}ClNO_2S_2$	60.93	3.90	3.38	61.03	3.71	3.15
10i	3-Methyl-2-thienyl	MeO	$C_{22}H_{19}NO_2S_2$	67.15	4.87	3.56	67.36	4.80	3.56
10j	3-Thienyl	MeO	$C_{21}H_{17}NO_2S_2$	66.47	4.52	3.69	66.72	4.40	3.69
10k	2-Pyrrolyl	MeO	$C_{21}H_{18}N_2O_2S$	69.59	4.96	7.73	70.06	4.90	7.68
10l	1,5-Dimethyl-2-pyrrolyl	H	$C_{21}H_{18}N_2S$	76.33	5.49	8.48	76.64	5.25	8.48
10m	1,5-Dimethyl-2-pyrrolyl	F	$C_{21}H_{16}F_2N_2S$	68.84	4.40	7.65	68.61	4.26	7.51
10o	1,5-Dimethyl-2-pyrrolyl	MeS	$C_{23}H_{22}N_2S_3$	65.37	5.25	6.63	65.55	5.28	6.48
10p	Pyrazinyl	MeO	$C_{21}H_{17}N_3O_2S$	67.18	4.56	11.19	67.47	4.49	11.31

TABLE VI. ^{1}H-NMR Spectra of 4,5-Diphenylthiazole Derivatives (**10**)

	R	R^3	^{1}H-NMR chemical shifts[a] (δ)
10a	3-Pyridyl	MeO	3.8 (6H, s), 6.8—6.95 (4H, m), 7.2—7.6 (5H, m), 8.2—8.35 (1H, m), 8.55—8.7 (1H, m), 9.15—9.25 (1H, m)
10b	2-Pyridyl	MeO	3.7 (6H, s), 6.6—6.9 (4H, m), 7.0—7.8 (6H, m), 8.0—8.25 (1H, m), 8.35—8.55 (1H, m)
10c	6-Methyl-2-pyridyl	MeO	2.6 (3H, s), 3.8 (6H, s), 6.65—7.7 (10H, m), 7.85—8.1 (1H, m)
10d	4-Methyl-2-pyridyl	MeO	2.4 (3H, s), 3.7 (6H, s), 6.7—7.6 (9H, m), 7.9—8.05 (1H, m), 8.2—8.35 (1H, m)
10e	6-Methoxy-2-pyridyl	MeO	3.75 (6H, s), 3.95 (3H, s), 6.55—6.9 (5H, m), 7.1—7.8 (6H, m)
10f	2-Thienyl	MeO	3.75 (6H, s), 6.6—7.55 (11H, m)
10g	5-Methyl-2-thienyl	MeO	2.45 (3H, s), 3.75 (6H, s), 6.55—6.9 (5H, m), 7.1—7.55 (5H, m)
10h	5-Chloro-2-thienyl	MeO	3.7 (6H, s), 6.6—6.85 (5H, m), 7.0—7.5 (5H, m)
10i	3-Methyl-2-thienyl	MeO	2.5 (3H, s), 3.75 (6H, s), 6.65—7.0 (5H, m), 7.1—7.6 (5H, m)
10j	3-Thienyl	MeO	3.75 (6H, s), 6.6—6.85 (4H, m), 7.1—7.55 (5H, m), 7.65—7.8 (1H, m)
10k	2-Pyrrolyl	MeO	3.7 (6H, s), 6.05—6.15 (1H, m), 6.5—6.9 (6H, m), 7.1—7.5 (4H, m), 9.4—9.8 (1H, br)
10l	1,5-Dimethyl-2-pyrrolyl	H	2.3 (3H, s), 4.0 (3H, s), 5.95 (1H, d, J=4.0), 6.6 (1H, d, J=4.0), 7.15—7.4 (8H, m), 7.5—7.7 (2H, m)
10m	1,5-Dimethyl-2-pyrrolyl	F	2.25 (3H, s), 3.95 (3H, s), 5.9 (1H, d, J=4.0) 6.55 (1H, d, J=4.0), 6.85–7.7 (8H, m)
10o	1,5-Dimethyl-2-pyrrolyl	MeS	2.3 (3H, s), 2.5 (6H, s), 4.0 (3H, s), 5.95 (1H, d, J=4.0), 6.6 (1H, d, J=4.0), 7.1—7.3 (6H, m), 7.4—7.5 (2H, m)
10p	Pyrazinyl	MeO	3.85 (6H, s), 6.8—6.95 (4H, m), 7.2—7.65 (5H, m), 8.5—8.6 (2H, m), 9.4—9.5 (1H, m)

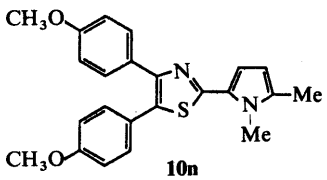
a) Measured in $CDCl_3$.

TABLE VII. *In Vitro* Platelet Aggregation Inhibitory Activity of 4,5-Diphenylazole Derivatives[a]

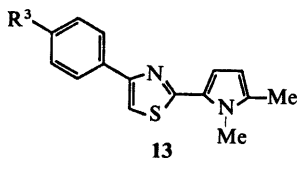
Imidazole (**4**)				Thiazole (**10**)			
	R	R^3	IC_{50}[b,c]		R	R^3	IC_{50}[b,c]
4a	3-Pyridyl	H	76.9				
4b	3-Pyridyl·H_2O	MeO	18.9	**10a**	3-Pyridyl	MeO	147
4c	2-Pyridyl	MeO	15.9	**10b**	2-Pyridyl	MeO	8.85
4d	6-Methyl-2-pyridyl	MeO	14.5	**10c**	6-Methyl-2-pyridyl	MeO	9.83
				10d	4-Methyl-2-pyridyl	MeO	7.50
4e	4-Pyridyl	MeO	2040	**10e**	6-Methoxy-2-pyridyl	MeO	33.9
4f	2-Thienyl	H	5140				
4g	2-Thienyl	MeO	20.0	**10f**	2-Thienyl	MeO	6.80
4h	5-Methyl-2-thienyl	MeO	720	**10g**	5-Methyl-2-thienyl	MeO	7.76
				10h	5-Chloro-2-thienyl	MeO	6.29
4i	3-Methyl-2-thienyl	MeO	9.42	**10i**	3-Methyl-2-thienyl	MeO	11.0
4j	3-Thienyl	MeO	60.4	**10j**	3-Thienyl	MeO	11.4
4k	2-Pyrrolyl	MeO	7.44	**10k**	2-Pyrrolyl	MeO	8.33
				10l	1,5-Dimethyl-2-pyrrolyl	H	3360
				10m	1,5-Dimethyl-2-pyrrolyl	F	66.4
4l	1,5-Dimethyl-2-pyrrolyl	MeO	86.5	**10n**	1,5-Dimethyl-2-pyrrolyl	MeO	6.42
				10o	1,5-Dimethyl-2-pyrrolyl	MeS	542
				10p	Pyrazinyl	MeO	18.5
	Aspirin		23200				

a) Rabbit PRP was used. b) Concentration of 4,5-bis(4-substituted-phenyl)azole required to inhibit 50% arachidonic acid-induced platelet aggregation. c) $\times 10^{-8}$ M. See Experimental section.

TABLE VIII. *In Vitro* Platelet Aggregation Inhibitory Activity of 4-(4-Substituted phenyl)thiazole Derivatives[a]



10n



13

	R^3	IC_{50}[b,c]
10n	—	6.42
13a	H	>10000
13b	MeO	>10000

a) Rabbit PRP was used. b) Concentration of 4-(4-substituted-phenyl)thiazole or 4,5-bis(4-substituted phenyl)thiazole required to inhibit 50% arachidonic acid-induced platelet aggregation. c) $\times 10^{-8}$ M. See Experimental section.

that of aspirin.

Cyclo-oxygenase Inhibitory Activity **10n**, which showed the strongest *ex vivo* platelet aggregation inhibitory activity in these diphenylazole derivatives was evaluated as cyclo-oxygenase inhibitory activity. The IC_{50} value of **10n** was 3.6×10^{-7} M. This result indicated that the mechanism of the activity of **10n** was the inhibition of cyclo-oxygenase.

Conclusion

In *in vitro* experiments, diphenylthiazole derivatives (**10**) were more potent than the corresponding diphenylimidazole derivatives (**4**) in inhibiting platelet aggregation of rabbit platelet-rich plasma. In both the imidazole and the thiazole series, removal or substitution of the methoxy groups at the 4-position of the benzene ring (**4a, f, 10 l, m, o**) resulted in a drop in the activity. 4-Phenylthiazole deriv-

TABLE IX. *Ex Vivo* Platelet Aggregation Inhibitory Activity of 4,5-Bis(4-methoxyphenyl)azole Derivatives[a]

	ED_{50}[b,c]	
	A.A.[d]	Coll.[e]
4i	>1.0	>1.0
4k	>1.0	>1.0
10b	>1.0	>1.0
10c	>1.0	>1.0
10d	0.50	1.14
10f	>1.0	>1.0
10g	>1.0	>1.0
10h	0.44	0.84
10k	>1.0	>1.0
10n	0.22	0.29
Aspirin	38.2	58.9

a) Guinea pig PRP was used. *b*) Dose of tested compound required to inhibit 50% platelet aggregation. *c*) mg/kg. See Experimental section. *d*) Arachidonic acid-induced platelet aggregation. *e*) Collagen-induced platelet aggregation.

atives (**13**) did not exhibit any inhibitory activity. Two diphenylimidazole derivatives (**4i, k**) and eight diphenylthiazole derivatives (**10b—d, f—h, k, n**) were evaluated for *ex vivo* experiments. 4,5-Bis(4-methoxyphenyl)-2-(1,5-dimethylpyrrol-2-yl)thiazole (**10n**) showed the strongest activity in *ex vivo* experiments. The activity of **10n** in *ex vivo* was 200 times stronger than that of aspirin. The mechanism of the activity of **10n** was the inhibition of cyclo-oxygenase.

Experimental

Anisil (**2**),[22] 4,5-diphenyl-2-(3-pyridyl)imidazole (**4a**),[21] 4,5-diphenyl-2-(2-thienyl)imidazole (**4f**),[21] pyridine-3-carbothioamide (**7a**),[25] pyridine-2-carbothioamide (**7b**),[26] 6-methylpyridine-2-carbothioamide (**7c**),[27] 4-methylpyridine-2-carbothioamide (**7d**),[28] thiophene-2-carbothioamide derivatives (**7f—i**),[29] thiophene-3-carbothioamide (**7j**),[30] pyrrole-2-carbothioamide derivatives (**7k, l**),[31] pyrazine-2-carbothioamide (**7m**),[32] bromodeoxyanisoin (**9a**),[23] α-bromo-4,4′-dimethylthiodeoxybenzoin (**9b**),[23] bromodeoxybenzoin (**9c**),[24] α-bromo-4,4′-difluorodeoxybenzoin (**9d**),[24] phenacyl bromide (**12a**),[35] and 4-methoxyphenacyl bromide (**12b**),[35] were prepared according to the procedures in the literatures. Melting points were taken on a capillary melting point apparatus (Yamato MR-21). All melting points were uncorrected. The structures of all compounds were supported by their infrared (IR) (Hitachi 270-50) and 60 and 100 MHz proton nuclear magnetic resonance (^{1}H-NMR) (Hitachi R-24A and Nihon Denshi PS-100) spectra. 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.

Anisil (2) To a solution of cupric sulfate pentahydrate (173 g, 694 mmol) in pyridine (260 ml) and water (86 ml), anisoin (**1a**) (91 g, 333 mmol) was added. The mixture was refluxed for 2 h. The solution was poured into ice water. The above mixture was acidified with concentrated HCl (300 ml). The precipitated crystals were separated by filtration, washed with water, dried, and then recrystallized from benzene to give 74.6 g (yield 83%) of **2** as green needles: mp 132.0—134.0 °C. NMR ($CDCl_3$) δ: 3.8 (6H, s), 6.9 (4H, m), 7.9 (4H, m).

4,5-Bis(4-methoxyphenyl)-2-(2-pyridyl)imidazole (4c) A mixture of anisil (**2**) (2.7 g, 10 mmol) and ammonium acetate (11.5 g, 150 mmol) in DMSO (60 ml) was heated to 100 °C. Pyridine-2-carboxaldehyde (**3c**) (4.3 g, 40 mmol) was dissolved in DMSO and was added dropwise to the above solution over 90 min. The solution was cooled to room temperature and poured into ice water and aqueous ammonia. The precipitated crystals were separated by filtration, washed with water, and then dissolved in ethyl acetate, and washed twice with water. The ethyl acetate layer was dried over anhydrous magnesium sulfate, and evaporated *in vacuo*. The residue was chromatographed on a silica gel column with benzene : ethyl acetate = 10 : 1 as eluent. Recrystallization from benzene gave 1.7 g (yield 48%) of **4c** as colorless needles: mp 190.0—191.0 °C. NMR ($CDCl_3$) δ: 3.9 (6H, s), 6.7—6.9 (4H, m), 7.1—7.9 (6H, m), 8.2—8.4 (2H, m), 10.4—11.3 (1H, br). *Anal.* Calcd for $C_{22}H_{19}N_3O_2$: C, 73.93; H, 5.36; N, 11.76. Found: C, 74.18; H, 5.30; N, 11.76.

4,5-Bis(4-methoxyphenyl)-2-(2-pyrrolyl)imidazole (4k) To a mixture of anisil (**2**) (3.0 g, 11 mmol) and pyrrole-2-carboxaldehyde (**3k**) (2.4 g, 25 mmol) in acetic acid (60 ml), was added ammonium acetate (12.8 g, 165 mmol). The mixture was refluxed for 90 min. The solution was cooled to room temperature and poured into ice water. The precipitated crystals were separated by filtration, washed with water, dried, and chromatographed on a silica gel column with benzene : ethyl acetate = 4 : 1 as eluent. Recrystallization from benzene gave 1.4 g (yield 37%) of **4k** as pale purple needles: mp 229.5—231.0 °C. NMR (DMSO-d_6) δ: 3.7 (6H, s), 5.9—6.1 (1H, m), 6.5—6.8 (6H, m), 7.1—7.4 (4H, m), 8.1—8.8 (1H, br). *Anal.* Calcd for $C_{21}H_{19}N_3O_2$: C, 73.03; H, 5.54; N, 12.17. Found: C, 73.22; H, 5.51; N, 11.98.

1,5-Dimethylpyrrole-2-carbothioamide (7l) 1,5-Dimethylpyrrole-2-carbonitrile (**6l**)[33] (24.0 g, 200 mmol) was dissolved in a solution of triethylamine (10.1 g, 100 mmol) in pyridine (30 ml), and therein hydrogen sulfide was blown at 20 °C for 8 h. The reaction mixture was diluted with water (600 ml), and the precipitated crystals were separated by filtration, washed with water, dried, and then recrystallized from ligroin to give 24.8 g (yield 81%) of 7l as a pale yellow powder: mp 95.0—99.0 °C. NMR ($CDCl_3$) δ: 2.3 (3H, s), 4.0 (3H, s), 5.9—6.1 (1H, m), 6.4—6.6 (1H, m), 6.6—6.8 (1H, m).

Bromodeoxyanisoin (9a) A solution of phosphorus tribromide (22.3 g, 82 mmol) and carbon tetrachloride (40 ml) was added over 2 h to a reflux solution of anisoin (**1a**) (56 g, 206 mmol) in carbon tetrachloride (1 l). The solution was cooled to room temperature and poured into ice water. The organic layer was diluted with ethyl acetate (2 l), dried over anhydrous magnesium sulfate, and evaporated *in vacuo*. The residue was recrystallized from benzene to give 61.4 g (yield 89%) of **9a** as a colorless powder: mp 101—103.5 °C. NMR ($CDCl_3$) δ: 3.7 (3H, s), 3.8 (3H, s), 6.3 (1H, s), 6.7—6.9 (4H, m), 7.2—7.55 (2H, m), 7.65—7.9 (2H, m).

4,5-Bis(4-methoxyphenyl)-2-(1,5-dimethyl-2-pyrrolyl)thiazole (10n) 1,5-Dimethylpyrrole-2-carbothioamide (7 l) (4.6 g, 30 mmol) and bromodeoxyanisoin (**9a**)[23] (10.1 g, 30 mmol) were dissolved in acetonitrile (300 ml). The mixture was stirred at 60 °C for 50 min. After cooling the solution, the reaction mixture was evaporated *in vacuo*. The resulting residue was dissolved in chloroform (300 ml) and an aqueous solution of sodium carbonate (300 ml), and the mixture was shaken. The chloroform layer was taken, and the aqueous layer was further extracted with chloroform (300 ml). The chloroform layers were combined, dried over anhydrous magnesium sulfate, and evaporated *in vacuo*. The residue was recrystallized from cyclohexane to give 8.8 g (yield 75%) of **10n** as colorless needles: mp 115.5—117.5 °C. NMR ($CDCl_3$) δ: 2.3 (3H, s), 3.7 (6H, s), 4.0 (3H, s), 5.9—6.1 (1H, m), 6.4—6.8 (5H, m), 7.0—7.5 (4H, m). *Anal.* Calcd for $C_{23}H_{22}N_2O_2S$: C, 70.74; H, 5.68; N, 7.17. Found: C, 70.78; H, 5.57; N, 6.97.

2-(1,5-Dimethyl-2-pyrrolyl)-4-phenylthiazole (13a) 1,5-Dimethylpyrrole-2-carbothioamide (7 l) (1.54 g, 10 mmol) and phenacyl bromide (**12a**) (1.99 g, 10 mmol) were dissolved in acetonitrile (30 ml). The mixture was refluxed for 3 h. After cooling the solution, the reaction mixture was evaporated *in vacuo*. The resulting residue was poured into water (100 ml). The precipitated solid was washed with an aqueous solution of sodium carbonate. The solid was recrystallized from hexane to give 2.00 g (yield 79%) of **13a** as yellow needles: mp 114—116 °C. NMR ($CDCl_3$) δ: 2.3 (3H, s), 4.0 (3H, s), 5.9 (1H, d), 6.6 (1H, d), 7.2 (1H, s), 7.3—7.5 (3H, m), 7.8—8.0 (2H, m). *Anal.* Calcd for $C_{15}H_{14}N_2S$: C, 70.84; H, 5.55; N, 11.01. Found: C, 70.82; H, 5.42; N, 10.71.

2-(1,5-Dimethyl-2-pyrrolyl)-4-(4-methoxyphenyl)thiazole (13b) 1,5-Dimethylpyrrole-2-carbothioamide (7 l) (1.54 g, 10 mmol) and 4-methoxyphenacyl bromide (**12b**) (2.29 g, 10 mmol) were dissolved in acetonitrile (30 ml). The mixture was refluxed for 3 h. After cooling the solution, the reaction mixture was evaporated *in vacuo*. The resulting residue was poured into water (100 ml). The precipitated solid was washed with an aqueous solution of sodium carbonate. The solid was recrystallized from cyclohexane to give 2.70 g (yield 95%) of **13b** as pale yellow scales: mp 174—176 °C. NMR ($CDCl_3$): 2.4 (3H, s), 3.8 (3H, s), 4.0 (3H, s), 5.9 (1H, d), 6.5 (1H, d), 6.8—7.2 (3H, s), 7.7—7.9 (2H, m). *Anal.* Calcd for $C_{16}H_{16}N_2OS$: C, 67.58; H, 5.67; N, 9.85. Found: C, 67.43; H, 5.51; N, 9.76.

Preparation of Platelet-Rich Plasma (PRP) Platelet-rich plasma was prepared from citrated blood by centrifugation at 150 × *g* for rabbit (*in vitro*) and guinea pig (*ex vivo*) for 10 min. After PRP was withdrawn, the residual blood was centrifuged at 2000 × *g* for 15 min and platelet-poor plasma (PPP) was obtained from the supernatant.

Pharmacological Assay for Platelet Aggregation Inhibition *in Vitro*

Experiments Platelet aggregation was performed by the turbine methods of Born[36] using the Platelet Aggregation Profiler PAP-3 (Bio Data Inc.). A 450 μl sample of test drugs or DMSO was incubated for 3 min at 37 °C. After incubation, platelet aggregation was induced by the addition of 50 μl of arachidonic acid (A.A.). Changes in the light transmission of PRP after the addition of arachidonic acid were recorded. The maximum increase in light transmission determined from the aggregation curve for 3 min was defined as percentage of aggregation. Percentage of inhibition of aggregation was calculated as

$$\% \text{ inhibition} = \left(1 - \frac{\% \text{ aggregation (drug)}}{\% \text{ aggregation (control)}}\right) \times 100$$

The concentration of tested drug which caused a 50% inhibition of aggregation was determined by linear regression analysis and represented the IC_{50}.

Ex Vivo **Experiments** Guinea pigs weighing 350—450 g were orally given a test drug or the vehicle. At the scheduled time after oral administration of the drug, blood was collected and platelet aggregation was measured by the same method as *in vitro* experiments. The aggregating agent was 0.1 mM (final concentration) of A. A. and 10 μg (final concentration) of collagen. The inhibitory effects of the drugs were determined by calculating the percentage inhibition with respect to the control value. The dose of tested drug which caused a 50% inhibition of aggregation (ED_{50}) was determined by linear regression.

Cyclo-oxygenase Inhibitory Activity Cyclo-oxygenase inhibitory activity was measured by the method of Miyamoto.[37] 0.1 ml of 100 mM Tris–HCl buffer (pH 8.0) containing 2 μM hematin, 1 mM 4-(hydroxymercuri)benzoic acid, 5 mM L-tryptophan, sheep seminal vesicular gland microsomes (1.0 mg/ml, final concentration), and drugs were pre-incubated for 2 min at 24 °C. The reaction was started by the addition of 1 nmol [^{14}C]-arachidonic acid (125000 dpm) and incubated for 2 min at 24 °C. The reaction was stopped by the addition of a mixture of ether, methanol, and 0.2 M citric acid (30 : 4 : 1) which was pre-cooled to −20 °C. The reaction mixture was mixed with 0.5 g of sodium sulfate. A 100 μl aliquot of the organic phase was placed on a pre-coated silica gel glass plate with PGB_2 as a standard. Thin-layer chromatography was carried out to a height about 17 cm at −20 °C using solvent system ether, petroleum ether, and glacial acetic acid (85 : 15 : 1). Dried plates were subjected to autoradiography with X-ray film and the location of PGH_2 was confirmed. The chromatograms were divided into appropriate zones and silica gel was scraped into counting vials. The radio activity was determined by an Aloka liquid scintillation spectrophotometer, model LSC 1050, in a toluene solution containing 0.03% 1,4-bis[2-(5-phenyloxazolyl)]benzen and 0.5% 2,5-diphenyloxazole. Cyclo-oxygenase inhibitory activity was measured as the synthesis of PGH_2 and IC_{50} value was calculated by linear regression.

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