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2,2′-Dithiodibenzamides as Inhibitors of Blood Platelet Aggregation

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New and known analogues of 2,2′-dithiodibenzamide were synthesized and tested for various pharmacological actions. This series of compounds was found to inhibit collagen- and adenosine 5′-diphosphate-induced aggregations of blood platelets. 2,2′-Dithiobis (*N*-(2-hydroxypropyl)benzamide) (**3**, KF4939) was one of the most potent compounds in *in vitro* aggregometry studies. Compound **3** after oral administration also potently inhibited pulmonary thrombosis induced by arachidonic acid injection in mice and platelet aggregation *ex vivo* in rats. This compound may have clinical value as a new orally active inhibitor of platelet aggregation.

Keywords——platelet aggregation inhibitor; dithiodibenzamide; structure–activity relationship; pulmonary thrombosis; *in vitro* aggregometry; *ex vivo* aggregometry

Since the chemical studies on 2,2′-dithiodibenzamides 1923,[1] related compounds have been synthesized by several different groups. Little is known of the biological activities of these analogues, though antifungal and anthelmintic effects were reported by Gialdi and his collaborators[2,3] and by Delacoux *et al.*,[4] respectively.

In order to find new biological activities of these analogues, we have synthesized many new analogues and carried out pharmacological screening tests. It was found that 2,2′-dithiodibenzamides possess antiaggregating activity towords blood platelets. We have already reported the inhibition of platelet aggregation by the most interesting compound, 2,2′-dithiobis(*N*-(2-hydroxypropyl)benzamide) (**3**, KF4939).[5] This paper deals with the inhibitory effects on platelet aggregation and the structure–activity relationship of a series of 2,2′-dithiodibenzamides.

Synthesis

The synthetic route to new analogues of 2,2′-dithiodibenzamides is shown in Chart 1. 2,2′-Dithiodibenzoic acid (I) was synthesized from anthranilic acid by the method of Allen and Mackay.[6] 2,2′-Dithiodibenzoyl chloride (II) was prepared by the chlorination of I with thionyl chloride in toluene in the presence of a catalytic amount of pyridine. Most of the 2,2′-dithiodibenzamides were synthesized by the reaction of II with 4 equivalents of amines or 2 equivalents of piperazine analogues in an inactive solvent such as tetrahydrofuran (THF) and/or dioxane under cooling. 2,2′-Dithiobis(*N*-hydroxymethylbenzamide) (**1**) was prepared from 2,2′-dithiodibenzamide and formalin in dimethylsulfoxide (DMSO). 2,2′-Dithiobis[(3-alkyloxypropylbenzamide)] derivatives were synthesized by the reaction of 2,2′-dithiobis(*N*-

(3-hydroxypropyl)benzamide) with the appropriate alkyl acid chloride in the presence of pyridine as a scavenger of the generated hydrochloric acid. Infrared (IR) spectra, elemental analysis and melting points were obtained for all new and known compounds and nuclear magnetic resonance (NMR) spectra were obtained for new compounds. The data were consistent with the assigned structures. Melting points, yields and elemental analysis data of new compounds are summarized in Table III, and their IR spectra and NMR spectra are described in Experimental.

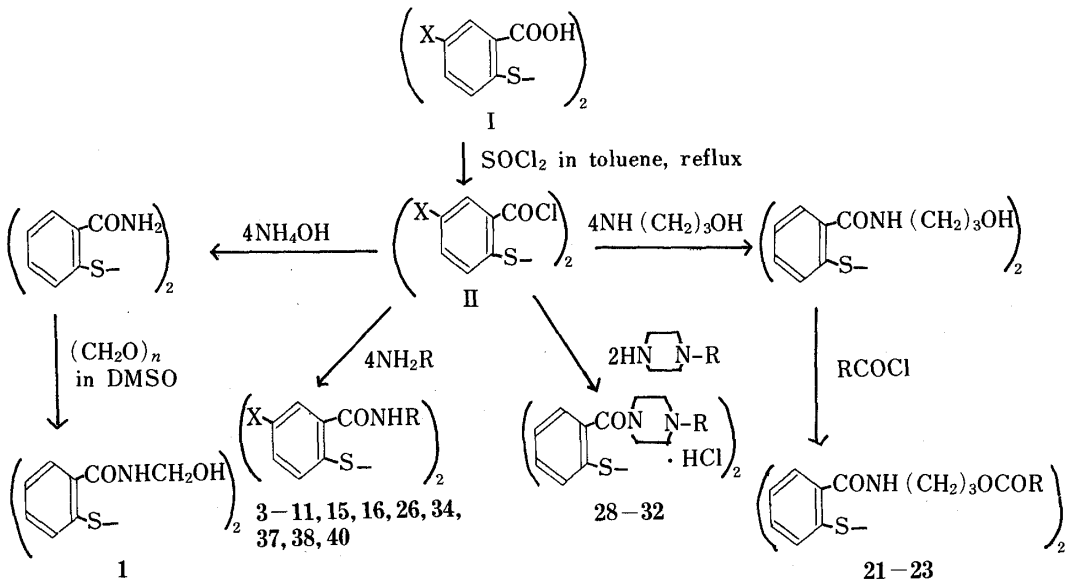


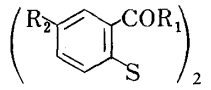
Chart 1

Pharmacological Studies

The compounds listed in Table I were evaluated for activity to inhibit collagen- and adenosine 5′-diphosphate (ADP)-induced aggregation of rabbit platelets. Among the compounds tested, **2**, **3**, **14**, **24** and **27** formed the most active class. These compounds showed effective inhibition (above 50%) of collagen- and ADP-induced aggregation at 3 μg/ml and 10 μg/ml, respectively. IC 50's (the concentration required to inhibit the induced aggregation by 50%) of these compounds were clearly lower than those of aspirin and papaverine. The second most active class, which showed effective inhibition at 10 and 30 μg/ml, included **1**, **5**, **10**, **13**, **25** and **26**. It is evident that *N*-substituents for highly active compounds were restricted to hydroxyalkyl, morpholine and piperazine analogues. Compounds with other *N*-substituents, alkyl, phenyl and cyclohexyl were less active or inactive. The structure–activity relationship of all compounds tested cannot be discussed conclusively, because the number of compounds was limited and the mode of action of this series of compounds has not yet been clarified. As regards *N*-hydroxyalkylbenzamides and their analogues alone, the following tendencies were recognized. Conversion of the hydroxyl group to alkyloxy (**2**→**12**, **14**→**15**) or acyloxy (**14**→**21**, **22**, **23**) weakened the potency. The introduction of a phenyl group at the alkyl group (**2**→**8**) was also deleterious. A similar effect of the phenyl group was also recognized in the case of piperazine analogues (**27**→**28**, **29**).

To evaluate the efficacy of compounds in the highly active class when given by oral administration, arachidonic acid-induced pulmonary thrombosis in mice and/or the *ex vivo* system in rats were employed. The data are shown in Table II. Of all the compounds tested, compound **3** exhibited the greatest inhibition in the two experiments. The inhibitory effects of compound **3** were comparable to those of aspirin, and the inhibitory effect in arachidonic acid-induced pulmonary thrombosis was stronger than those of dipyridamole and adenosine.

TABLE I. Inhibitory Effects of 2,2′-Dithiodibenzamides on Platelet Aggregation in Rabbit Platelet-Rich Plasma



Compd. No.	R_1	R_2	Per cent inhibition of platelet aggregation									
			Collagen					ADP				
			100[b]	30	10	3	IC50[c] μg/ml	100	30	10	3	IC50 μg/ml
1	$NHCH_2OH$	H		100	56	22			78	60	34	
2[a]	$NH(CH_2)_2OH$	H			100	67	2.7	100	93	55	34	5.5
3	$NHCH_2CH(OH)CH_3$	H			100	84	2.9		90	54	26	8.0
4	$NHCH(CH_2CH_3)CH_2OH$	H	71	17				62	10			
5	$NHCH_2CH(OH)C_2H_5$	H	100	70	50			100	55			
6	$NHC(CH_3)_2CH_2OH$	H	100	30	0			97	25			
7	$NHCH(CH(CH_3)_2)CH_2OH$	H	70	20				10				
8	$NHCH_2CH(OH)$–Ph	H	0					0				
9	$NHCH(CH_3)CH(OH)$–Ph	H	68	35	0			32	15			
10	$NH(CH_2)OH$	Cl				100		100	80	43	0	
11	$NHCH_2CH(OH)CH_3$	CH_3		100	32			98	45	21		
12[a]	$NH(CH_2)_2CH_3$	H		100	40			75				
13	$NHCH_2CH(OCH_3)_2$	H		100	87	21		100	83	35	21	
14[a]	$NH(CH_2)_3OH$	H		100	78	50	2.9		88	64	42	5.3
15	$NH(CH_2)_3OC_2H_5$	H	100	31	15			35				
16	$NH(CH_2)_4OH$	H		100	97	20		100	45			
17[a]	$NHCH_2CH=CH_2$	H	100	98	16			82	41			
18[a]	$NH(CH_2)_3CH_3$	H		100	13			47	13			
19[a]	$NH(CH_2)_9CH_3$	H	89	0				35	0			
20[a]	$NH(CH_2)_{11}CH_3$	H	0					0				
21	$NH(CH_2)_3COOCH_3$	H		100	14			83	46			
22	$NH(CH_2)_3COO(CH_2)_4CH_3$	H	100	29				75	38			
23	$NH(CH_2)_3COO(CH_2)_8CH_3$	H	11					10				
24[a]		H			100	59			100	60	40	
25[a]	NH– 	H			100	40		100	56	0		
26	$NH(CH_2)_3$– 	H		100	98	21			100	69	26	
27[a]		H		100	90	59	4.9		100	62	26	5.2
28		H		100	47	8		100	83	47	24	
29		H	90	22	0			37	16			
30		H		100	55	0			43	32		
31		H	100	40	23			95	17			
32		H		99	45	14			55	19		
33[a]	NH–Ph	H	86	57				54	26			
34	NH–Ph–*o*-NO_2	H	90	45				50	20			
35[a]	NH–Ph–*o*-Cl	H	100	63	4			96	24	0		
36[a]	NH–Ph–*m*-Cl	H	100	99	0			96	29	0		
37	NH–Ph–*m*-CF_3	H	56					33				
38	NH–Ph–*p*-CH_2COOH	H	21					0				
39[a]	NH–cyclohexyl	H	38	25				36	15			
40	NH–cyclohexyl–*p*-OH		95	8				35				
	Aspirin						300					300<
	Papaverine						41					51

a) Known compound. *b*) Concentration of compound (μg/ml). *c*) The IC50 value was obtained from the mean inhibition % of 3—4 experiments.

TABLE II. Anti-platelet Effects of 2,2′-Dithiodibenzamides Given by Oral Administration

Compd. No.	Arachidonate-induced pulmonary thrombosis		Collagen-induced platelet aggregation *ex vivo* in rats		Acute lethality of mice[d] died/used
	Dose mg/kg	Mortality of mice[a] died/used	Dose mg/kg	Inhibition %[c]	
1	N.T.[b]		100	50.1	0/3
2	50	10/14	50	24.2	0/3
3	50	4/20 $p<0.01$[e]	50	65.5 $p<0.05$	0/10
10	50	6/10	50	67.5 $p<0.05$	0/3
12	50	6/10	N.T.		0/3
14	50	6/10	50	33.9	0/3
24	50	7/10	50	21.6	0/3
26	50	9/20	N.T.		0/3
27	N.T.		50	0	3/3
28	N.T.		100	27.5	0/3
Aspirin	50	1/24 $p<0.01$	50	80.5 $p<0.01$	
Dipyridamole	100	17/30	N.T.		
Adenosine	100	13/14	N.T.		

a) The mortality in the vehicle-treated group was 69.8% (67/96). *b*) Not tested. *c*) The mean aggregation rate in the vehicle-treated group was taken as 100%. Five to six rats were used for each group. *d*) The dose of compounds was 1000 mg/kg *p.o.* *e*) Significantly different from control group.

Compound **10** showed comparable inhibition to **3** in the *ex vivo* system. When compound **3** or **10** at 1000 mg/kg was orally administered to mice, no death was observed. Therefore, these compounds have weak toxicity. We consider that compound **3** is worthy of further evaluation as a new orally active antithrombotic agent.

Experimental

Melting points were determined in open capillaries in a Shibata melting point apparatus and are uncorrected. IR spectra were recorded on a Hitachi 260-30 IR instrument. NMR spectra were recorded on a JEOL JNM-PMX-60 NMR spectrometer using Me_4Si as an internal standard.

Chemistry

Preparation of 2,2′-Dithiobis(*N*-hydroxymethylbenzamide) (1)——Formalin (1.6 g, 20 mmol; as 37% aqueous formaldehyde solution) was added dropwise to a solution of 2,2′-dithiodibenzamide (3.0 g, 10 mmol) in 30 ml of DMSO with stirring at room temperature. Then, two drops of 1 N NaOH were added. After the addition, the mixture was stirred for 1 h at room temperature and for an additional 2 h at 65—70 °C. After cooling to room temperature, the mixture was poured into 300 ml of cold water. The solution was allowed to stand overnight at room temperature, yielding a white crystalline precipitate. The precipitate was removed by filtration, washed thoroughly with water, and dried to give 3.0 g of **1**. IR (KBr) cm^{-1}: 3920 (NH), 2950 (CH_2), 1635 (C=O). ^{1}H-NMR (DMSO-d_6) δ: 9.30 (2H, t, NH), 7.3—8.9 (8H, m, Ph), 5.86 (2H, s, OH), 4.83 (4H, s, $NHCH_2OH$).

Preparation of 2,2′-Dithiobis(*N*-2-hydroxypropylbenzamide) (3)——2,2′-Dithiodibenzoyl chloride (10.3 g, 30 mmol) was suspended in 50 ml of dioxane and the mixture was cooled to 10—13 °C in an ice bath. To this solution, a solution of 2-hydroxypropylamine (9.0 g, 0.12 mol) in 50 ml of dioxane was added dropwise over 1 h with stirring. After the addition, the reaction mixture was held at room temperature for 2 h. After the completion of the reaction, the mixture was poured into 300 ml of ice water with stirring to form a white precipitate. The mixture was filtered and the precipitate obtained was dried. Recrystallization from EtOH yielded 10.1 g of **3**. IR (KBr) cm^{-1}: 3290 (NH), 2975 (CH_2), 1620 (C=O). ^{1}H-NMR (DMSO-d_6) δ: 8.53 (2H, t, NH), 7.2—7.8 (8H, m, Ph), 4.76 (2H, OH), 3.73 (m, CH), 3.20 (t, NCH_2), 1.10 (d, CH_2).

Compounds **4**—**11** and **16** were prepared in a similar manner using the appropriate hydroxyamines. Compound **4**: IR (KBr) cm^{-1}: 3290 (NH), 2970 (CH_3), 2940 (CH_2), 1620 (C=O). ^{1}H-NMR (DMSO-d_6) δ: 8.20 (2H, d, NH), 7.17—7.73 (8H, m, Ph), 3.66—4.10 (2H, br, OH), 3.50 (4H, CH_2), 1.67—1.83 (4H, m, $C\underline{H}_2CH$), 0.96 (6H, t, $CH_2C\underline{H}_3$). Compound **5**; IR (KBr) cm^{-1}: 3290 (NH), 2965 (CH_3), 2940 (CH_2), 1635 (C=O). ^{1}H-NMR (DMSO-d_6)

TABLE III. 2,2′-Dithiodibenzamides

Compd. No.	Yields (%)	mp (°C)	Recrystn. solvent	Formula	Analysis (%) Calcd (Found)		
					C	H	N
1	82.5	181 (dec.)	EtOH	$C_{16}H_{16}N_2O_4S_2$	52.73	4.43	7.69
					(52.77	4.31	7.69)
3	80.1	176—179	EtOH	$C_{20}H_{24}N_2O_4S_2$	57.12	5.75	6.66
					(56.89	5.73	6.67)
4	78.0	202—208	MDG	$C_{22}H_{28}N_2O_4S_2$	58.90	6.29	6.24
					(58.92	6.18	6.14)
5	76.0	169—171	IPA	$C_{22}H_{28}N_2O_4S_2$	58.90	6.29	6.24
					(58.88	6.30	6.20)
6	74.3	189—192	IPA	$C_{22}H_{28}N_2O_4S_2$	58.90	6.29	6.24
					(58.52	6.11	6.22)
7	57.4	189—192	MeOH–H_2O	$C_{24}H_{32}N_2O_4S_2$	60.48	6.77	5.88
					(59.89	6.52	5.89)
8	84.5	214—217	DMF–MeOH	$C_{30}H_{28}N_2O_4S_2$	66.15	5.18	5.14
					(65.91	5.18	5.09)
9	52.5	197—200	Acetone	$C_{32}H_{32}N_2O_4S_2$	67.11	5.63	4.89
					(67.01	5.60	4.59)
10	76.1	215—218	MeOH	$C_{18}H_{18}Cl_2N_2O_4S_2$	46.86	3.93	6.07
					(46.77	3.76	5.84)
11	33.4	160—164	EtOH	$C_{22}H_{28}N_2O_4S_2$	58.90	6.29	6.24
					(58.76	6.33	6.22)
13	62.3	135—136	MeOH–acetone	$C_{22}H_{28}N_2O_6S_2$	54.98	5.87	5.83
					(54.92	6.11	5.88)
15	73.2	125—126	MeOH–acetone	$C_{24}H_{32}N_2O_4S_2$	60.48	6.77	5.88
					(60.56	6.73	5.80)
16	58.1	137—139	MeOH–H_2O	$C_{22}H_{28}N_2O_4S_2$	58.90	6.29	6.24
					(58.75	6.39	6.52)
21	78.9	127—129	IPA	$C_{24}H_{28}N_2O_6S_2$	57.13	5.59	5.55
					(57.01	5.63	5.54)
22	66.9	107—108.5	Ethylacetone	$C_{32}H_{44}N_2O_6S_2$	62.31	7.19	4.54
					(62.11	7.00	4.68)
23	46.6	92.1—95.2	MeOH	$C_{40}H_{60}N_2O_6S_2$	65.90	8.30	3.84
					(65.22	8.40	3.80)
26	38.3	147—150	Benzene	$C_{28}H_{38}N_4O_4S_2$	60.16	6.85	10.03
					(60.51	7.01	9.85)
28	80.0	>230	EtOH	$C_{36}H_{38}N_4O_2S_2 \cdot 2HCl$	62.14	5.80	8.05
					(61.30	5.85	8.15)
29	85.0	215—216	EtOH	$C_{48}H_{46}N_4O_2S_2 \cdot 2HCl$	67.99	5.71	6.61
					(67.58	5.94	6.02)
30	78.2	122—125	EtOH	$C_{42}H_{50}N_4O_4S_2 \cdot 2HCl \cdot 2H_2O$	59.49	6.66	6.61
					(58.45	6.36	6.58)
31	56.3	>230	EtOH	$C_{40}H_{42}N_4O_2S_2 \cdot 2HCl$	64.24	5.93	7.49
					(63.81	5.76	7.21)
32	96.3	120—122	EtOH	$C_{34}H_{34}N_4O_2S_2 \cdot 2HCl$	61.16	5.44	8.39
					(60.58	5.43	8.21)
34	76.1	207—210	MeOH	$C_{26}H_{18}N_4O_6S_2$	57.14	3.32	10.25
					(56.99	3.15	10.13)
37	84.4	168—172	Benzene	$C_{28}H_{18}F_6N_2O_2S_2$	57.76	3.06	4.73
					(55.99	3.02	4.71)
38	83.2	>230	DMF	$C_{30}H_{24}N_2O_6S_2$	62.92	4.22	4.89
					(62.53	4.39	4.96)
40	93.3	234	MeOH	$C_{26}H_{32}N_2O_4S_2$	62.38	6.44	5.59
					(62.27	6.73	5.80)

δ: 8.43 (2H, t, NH), 7.17—7.69 (8H, m, Ph), 4.66 (2H, d, OH), 3.0—3.82 (6H, m, CH_2CH), 1.17—1.83 (4H, m, $C\underline{H}_2CH_3$), 0.96 (6H, t, CH_3). Compound **6**; IR (KBr) cm^{-1}: 3290 (NH), 2990 (CH_3), 2940 (CH_3), 2940 (CH_2), 1630 (C=O). ^{1}H-NMR (DMSO-d_6) δ: 7.83 (2H, s, NH), 4.83 (2H, t, OH), 3.53 (4H, d, $C\underline{H}_2OH$), 1.36 (12H, s, CH_3). Compound **7**; IR (KBr) cm^{-1}: 3290 (NH), 2980 (CH_3), 2880 (CH), 1630 (C=O). ^{1}H-NMR (DMSO-d_6) δ: 8.17 (2H, d, NH), 7.17—7.83 (8H, m, Ph), 0.96 (12H, d, CH-$\underline{Me}_2$). Compound **8**; IR (KBr) cm^{-1}: 3250 (NH), 1620 (C=O). ^{1}H-NMR (DMSO-d_6) δ: 8.66 (2H, t, NH), 7.16—7.83 (18H, m, Ph), 5.56 (2H, d, OH), 3.50 (6H, t, CH_2CH–). Compound **9**; IR (KBr) cm^{-1}: 3290 (NH), 3070 (CH_3), 2975 (CH), 1625 (C=O). ^{1}H-NMR (DMSO-d_6) δ: 8.40 (2H, d, NH), 7.10—7.73 (18H, m, Ph), 5.73 (2H, t, OH), 1.17 (6H, d, CH-$C\underline{H}_3$). Compound **10**; IR (KBr) cm^{-1}: 3290 (NH), 2940 (CH_2), 1635 (C=O). ^{1}H-NMR (DMSO-d_6) δ: 8.66 (2H, t, NH), 7.33—7.89 (6H, m, Ph), 3.00—4.00 (8H, m, CH_2CH_2). Compound **11**; IR (KBr) cm^{-1}: 3290 (NH), 2980 (CH_3), 2925 (CH_2), 1635 (C=O). ^{1}H-NMR (DMSO-d_6) δ: 8.40 (2H, t, NH), 7.07—7.20 (6H, m, Ph), 3.83 (2H, q, $C\underline{H}$–OH), 3.20 (4H, t, $NHC\underline{H}_2$), 2.30 (6H, s, CH_3), 1.17 (6H, d, $CHC\underline{H}_3$). Compound **16**; IR (KBr) cm^{-1}: 3250 (br, NH), 1620 (C=O). ^{1}H-NMR (DMSO-d_6) δ: 8.58 (2H, t, NH), 7.10—7.83 (8H, m, Ph), 4.41 (2H, t, OH), 3.36 (8H, t, $-NHC\underline{H}_2CH_2CH_2C\underline{H}_2OH$), 1.56 (8H, t, $CH_2C\underline{H}_2C\underline{H}_2CH_2OH$).

Preparation of 2,2′-Dithiobis(*N*-(2,2-diethoxyethyl)benzamide) (13)——2,2-Dimethoxyethylamine (6.32 g, 60 mmol) in 30 ml of dioxane was added dropwise to a suspension of 2,2′-dithiodibenzoyl chloride (5.15 g, 15 mmol) in 30 ml of dioxane over 1 h at 11—13 °C. Then the mixture was stirred at 40 °C for 2 h. On completion of the reaction, the solution was poured into cold water. The mixture was filtered and the precipitate obtained was washed thoroughly with water and dried. Upon recrystallization from acetone, **13** was precipitated slowly as white crystals. The yield was 4.5 g (62.3% of the theoretical amount). IR (KBr) cm^{-1}: 3290 (NH), 3050 (CH_3), 2950 (CH_2), 1630 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 7.17—7.86 (8H, m, Ph), 6.46 (2H, t, NH), 4.46 (2H, t, CH), 3.36 (12H, s, $(OCH_3)_2$).

Compound **15** was prepared in a similar manner. IR (KBr) cm^{-1}: 3290 (NH), 3050 (CH_3), 2970 (CH_2), 1620 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 7.86—7.66 (8H, m, Ph), 2.00 (4H, q, $NHCH_2C\underline{H}_2CH_2$), 1.20 (6H, t, $CH_2C\underline{H}_3$).

Preparation of 2,2′-Dithiobis[*N*-(3-acetoxypropyl)benzamide] (21)——A solution of acetyl chloride (7.9 g, 0.101 mol) in 50 ml of dichloromethane was added dropwise over 30 min to a solution of 2,2′-dithiobis(*N*-(3-hydroxypropyl)benzamide) (21 g, 0.05 mol) in 100 ml of dichloromethane with stirring at below 20 °C. The mixture was stirred for an additional 30 min. Then, pyridine (7.96 g, 0.101 mol) was added to the mixture over 1 h at below 20 °C. After the addition, stirring was continued for 2 h. The organic layer was extracted and the extract was washed with 200 ml of saturated salt solution, then dried over anhydrous magnesium sulfate. The solvent was evaporated off under reduced pressure. The solid obtained was recrystallized from isopropylalcohol to give 19.9 g (78.9%) of **21**. IR (KBr) cm^{-1}: 3300 (NH), 3070 (CH_3), 2975 (CH_2), 1735 (O–C=O). ^{1}H-NMR ($CDCl_3$) δ: 7.13—7.83 (8H, m, Ph), 6.73—7.10 (2H, t, NH), 4.17 (4H, t, $C\underline{H}_2$–O), 3.50 (4H, q, $NHC\underline{H}_2CH_2$), 2.03 (6H, s, CH_3).

Compounds **22** and **23** were prepared in a similar manner. Compound **22**; IR (KBr) cm^{-1}: 3330 (NH), 1730 (O–C=O), 1635 (NHC=O). ^{1}H-NMR ($CDCl_3$) δ: 7.00—7.80 (8H, m, Ph), 6.66—7.07 (2H, t, NH), 4.15 (4H, t, $C\underline{H}_2O$), 3.46 (4H, q, $NHC\underline{H}_2$), 0.75 (6H, t, $C\underline{H}_3$). Compound **23**; IR (KBr) cm^{-1}: 3275 (NH), 1730 (O–C=O), 1625 (NHC=O). ^{1}H-NMR ($CDCl_3$) δ: 7.00—7.89 (8H, m, Ph), 6.73 (2H, t, NH), 4.20 (4H, t, $C\underline{H}_2O$), 3.50 (4H, q, $NHCH_2$).

Preparation of 2,2′-Dithiobis[*N*-(3-morpholinopropyl)benzamide] (26)——A solution of *N*-(3-aminopropyl)-morpholine (8.7 g, 0.06 mol) in 30 ml of dioxane was added slowly over 1.5 h to a solution of 2,2′-dithiodibenzoyl chloride (10.3 g, 0.02 mol) in 100 ml of dioxane at below 20 °C. After the completion of the addition, the mixture was continuously stirred for approximately 2 h at room temperature. When the reaction was complete, the mixture was poured into a sodium bicarbonate solution, and the mixture was filtered with suction. The white precipitate obtained was thoroughly washed with water and dried. Then, recrystallization from benzene yielded 6.4 g (38.3%) of **26** as white crystals. IR (KBr) cm^{-1}: 3250 (NH), 2940 (CH), 1625 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 8.00 (2H, t, NH), 7.00—7.90 (8H, m, Ph), 3.57 (8H, t, $NHC\underline{H}_2CH_2C\underline{H}_2NH$), 1.81 (4H, q, $NHCH_2C\underline{H}_2CH_2N$<).

Preparation of 2,2′-Dithiobis(*N*′-benzylbenzpiperazide)hydrochloride Salt (28)——2,2′-Dithiodibenzoyl chloride (6.9 g, 0.02 mol) was dispersed in 50 ml of dioxane and the mixture was cooled to 11 °C in an ice-bath. To this mixture, a solution of benzylpiperazine (7.0 g, 0.04 mol) in 50 ml of dioxane was added slowly over 30 min. The mixture was stirred at 10 °C for 2 h, then filtered. The filtrate was washed with acetone and recrystallized from EtOH to give 11.1 g (80.0%) of **28** as white crystals. IR (KBr) cm^{-1}: 3400—3500 (br, $\rangle$N–CH_2), 2650 (CH_2), 2550, 2460 ($N\langle N\cdot HCl$).

Compounds **29**—**32** were prepared in a similar manner. Compound **29**; IR (KBr) cm^{-1}: 2550, 2450 ($N\langle N\cdot HCl$), 1630 (C=O). Compound **30**; IR (KBr) cm^{-1}: 3400 (br, $N\langle N$), 2550 ($N\langle N\cdot HCl$), 1630 (C=O). ^{1}H-NMR ($CDCl_3$) δ: 7.06—7.66 (18H, m, Ph), 5.33 (4H, br, OCH_2), 1.24 (6H, t, CH_2CH_3). Compound **31**; IR (KBr) cm^{-1}: 2500 (br, $N\langle N\cdot HCl$), 1635 (C=O). Compound **32**; IR (KBr) cm^{-1}: 1630 (C=O), 1600, 1500 (Ph). ^{1}H-NMR ($CDCl_3$) δ: 6.66—7.81 (18H, m, Ph), 3.1 (2H, br, NH).

Preparation of 2,2′-Dithiobis(*p*-hydroxycyclohexylbenzamide) (40)——*p*-Hydroxycyclohexylamine (6.91 g, 60 mmol) in 30 ml of dioxane was added dropwise to a suspension of 2,2′-dithiodibenzoyl chloride (5.15 g, 15 mmol)

in 50 ml of dioxane over 1 h at 11—13 °C. The mixture was stirred at 40 °C for 2 h. The salt of the amine was removed by filtration, the filtrate was evaporated *in vacuo*, and the residue was taken up in MeOH (100 ml). Water was added to this solution, and the precipitate obtained was filtered off. The solid was recrystallized from MeOH to give 7.5 g (93.3%) of **40**. IR (KBr) cm^{-1}: 3280 (NH), 1620 (C=O).

Compounds **34**, **37** and **38** were prepared in a similar manner. Compound **34**; IR (KBr) cm^{-1}: 1665 (C=O), 1340 ($-NO_2$). Compound **37**; IR (KBr) cm^{-1}: 1642 (C=O). Compound **38**; IR (KBr) cm^{-1}: 1680 (COOH), 1640 (NHC=O).

Compounds **2**,[7] **12**,[7] **14**,[7] **17**,[8] **18**,[8] **19**,[9] **20**,[2] **25**,[8] **27**,[10] **33**,[8] **35**,[4] **36**,[2] **39**[2] were prepared according to the reported methods.

Pharmacological Studies

Preparation of Platelet-Rich Plasma (PRP)——PRP was prepared from rabbit blood withdrawn into plastic tubes containing 3.8% sodium citrate (1 ml of citrate/9 ml of blood). PRP was obtained from the citrated blood by centrifugation at 1000 rpm for 15 min. Then, platelet-poor plasma (PPP) was obtained from the residual blood by centrifugation at 3000 rpm for 10 min. Following the centrifugation, PRP was stored at room temperature and was used in aggregometry studies between 30 and 180 min after collection.

***In Vitro* Aggregometry**——Platelet aggregation was measured turbidimetrically using a 3-channel aggregometer (Rikadenki, RAM-31) at 37 °C under stirring at 1100 rpm. Platelet aggregation was initiated by adding 10 μl of an aggregating agent, ADP (final conc., 10 μM) or collagen (bovine Achilles tendon, Sigma) suspension, to 0.25 ml of PRP. Collagen (500 mg) was homogenized in a glass homogenizer with Tyrode solution (50 ml) and the homogenate was centrifuged at 2500 rpm for 5 min to obtain supernatant suspension. A fine suspension was obtained from the supernatant by filtration through gauze. Test compound (10 μl) was added to the PRP 2 min before addition of the aggregating agent. Inhibition of platelet aggregation was assessed by comparing the maximal optical density changes of the compound-treated PRP sample and the vehicle-treated sample.

***Ex Vivo* Aggregometry in Rats**——Male Wistar strain rats weighing 210—230 g were used after overnight fasting. The test compounds were administered by gastric intubation. Two hours later, all animals were anesthetized with pentobarbital Na (50 mg/kg, *i.p.*). A catheter for blood sampling was placed in the left carotid artery. Blood (4.5 ml) was withdrawn into a polyethylene syringe containing 0.5 ml of 3.8% sodium citrate solution. PRP was obtained as described above and used for *in vitro* aggregometry studies.

Arachidonic Acid-Induced Pulmonary Thrombosis in Mice——The method used has been described previously.[11] Male ddY strain mice weighing 22—25 g were used. The compounds were administered by gastric intubation. One hour later, arachidonic acid (Sigma) at a dose of 50 mg/kg was injected into the tail vein. The mortality in each group was observed up to 2 h after the injection.

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