7-(Ethoxycarbonyl)-6,8-dimethyl-2-phenyl-1(2H)-phthalazinone Derivatives: Synthesis and Inhibitory Effects on Platelet Aggregation

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7-(Ethoxycarbonyl)-6,8-dimethyl-2-phenyl-1(2H)-phthalazinone derivatives and several analogues were synthesized and their inhibitory effects on platelet aggregation were evaluated. Structure–activity relationships are discussed. All synthesized compounds showed no appreciable effect on platelet aggregation induced by adenosine diphosphate, but most of them inhibited effectively the arachidonic acid induced platelet aggregation. The parent compound, 2-phenyl derivatives, and ortho-substituted 2-phenyl derivatives show the most potent inhibition of all compounds.

We reported earlier[1] the synthesis of 7-(ethoxy-carbonyl)-4-(hydroxymethyl)-6,8-dimethyl-2-phenyl-1-(2H)-phthalazinone derivatives (1), which showed fairly potent inhibitory activity on platelet aggregation induced by arachidonic acid. We have also found 7-(ethoxy-carbonyl)-6,8-dimethyl-2-phenyl-1(2H)-phthalazinone derivatives (2), having no substituent at position 4, exhibited more potent antiaggregating activity than 1. Some derivatives of 2 have been obtained as byproducts when synthesis of 1 was carried out via the reaction of the corresponding keto carboxylic acid with phenylhydrazine as described before.[1] By the inspection of CPK molecular models, it appeared that the molecular shape of 2 is similar to that of papaverine. Papaverine is a well-known vasodilator and also is reported to have inhibitory activity on platelet aggregation.[2] To investigate the relationship between similarity of molecular shapes and biological activities, several 2-phenyl-1(2H)-phthalazinone derivatives having two methoxy groups at positions 6 and 7 were also prepared.
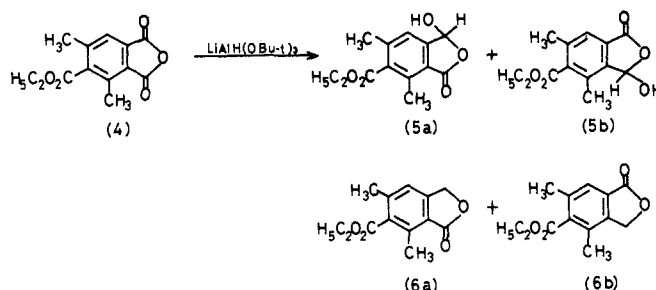
We report herein the syntheses of 6,7-dimethoxy-2-(substituted-phenyl)-1(2H)-phthalazinone (3) and 7-(ethoxycarbonyl)-6,8-dimethyl-2-(substituted-phenyl)-1-(2H)-phthalazinone derivatives (2) and the structure–activity relationship of these compounds with respect to antiaggregating activity.
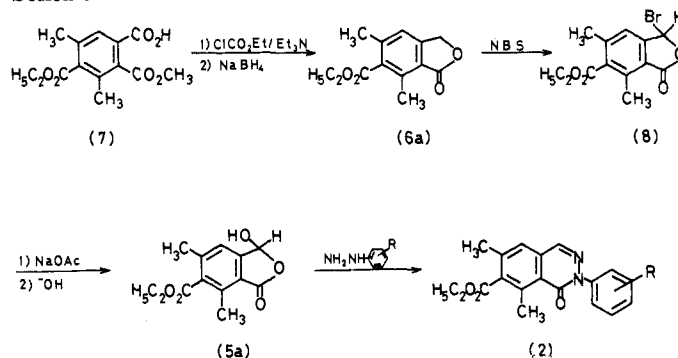
1, R′ = CH₂OH
2, R′ = H
3
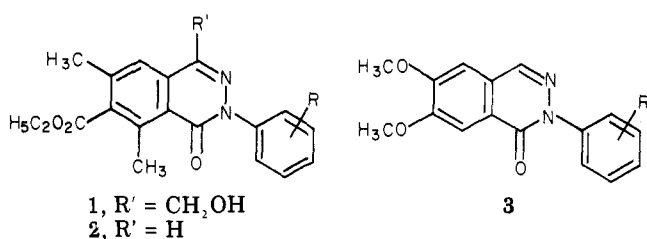
Chemistry. The synthesis of 6,7-dimethoxy-1(2H)-phthalazinone (3a) through the reaction of 2-formyl-4,5-dimethoxybenzoic acid (m-opianic acid) with phenyl-hydrazine had been reported by Fargher et al.[3] According to their method, the derivatives having a substituent on the 2-phenyl group of 3a were prepared, and the results are shown in Table I. These compounds were subjected to platelet aggregation and blood vessel relaxing tests. However, all of them did not inhibit the platelet aggregation induced by either ADP or arachidonic acid at a concentration of 100 μM. They also failed to show a relaxing effect on rabbit thoracic aorta contracted with KCl.

As described above, 2a had been obtained as byproduct in 15% yield when 4-carboxy-7-(ethoxycarbonyl)-6,8-dimethyl-1(2H)-phthalazinone was prepared. The other synthetic routes to obtain sufficient quantities of 2 were
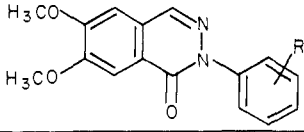
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Scheme I

(4)   (5a)   (5b)

(6a)   (6b)

Scheme II

(7)   (6a)   (8)

1) NaOAc
2) ⁻OH

(5a)   (2)

examined. It was assumed that the best intermediate to prepare the derivatives of 2 bearing a substituent on the 2-phenyl group was 6-(ethoxycarbonyl)-5,7-dimethyl-3-hydroxyphthalide (5a). The reduction of substituted phthalic anhydrides with various agents to the corresponding 3-hydroxyphthalides was reported previously.[4,5] It was expected that the reduction of 4 with lithium tri-tert-butoxyaluminum hydride (LiAlH(OBu-t)₃) proceeded regioselectively in the less sterically hindered carbonyl group at the 3-position. However, as shown in Scheme I, the reduction did not proceed with satisfactory regiospecificity. The reaction of 4 with LiAlH(OBu-t)₃ afforded a complex oily mixture, which could be separated by column chromatography into a mixture of two isomeric 3-hydroxyphthalides (5a, 5b; 48.9%) and a mixture of two isomeric phthalides (6a, 6b; 41.8%). Rechromatography of the former mixture on a silica gel column gave pure
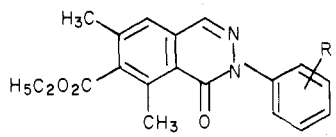
(1) Sugimoto, A.; Ito, S.; Eguchi, Y.; Tanaka, H.; Takashima, Y.; Ishikawa, M. *Eur. J. Med. Chem.*, submitted for publication.
(2) Mills, D. C. B.; Smith, J. B. *Biochem. J.* **1971**, *121*, 185.
(3) Fargher, R. G.; Perkin, W. H., Jr. *J. Chem. Soc.* **1921**, 1724.
(4) Taub, D.; Girotra, N. N.; Hoffsommer, R. D.; Kuo, C. H.; Slates, H. L.; Weber, S.; Wendler, N. L. *Tetrahedron* **1968**, *24*, 2443.
(5) McAlees, A. J.; McCrindle, R.; Sneddon, D. W. *J. Chem. Soc., Perkin Trans. 1* **1977**, 2030.

Table I. Physical Constants and Antiaggregation Activities of 6,7-Dimethoxy-2-(substituted-phenyl)-1(2H)-phthalazinone (3)

3	R	mp, °C (recrystn solvent)	formula[a]	yield, %	inhibn of AA-induced platelet aggregation: ED_{50}, μM
a	H	227–228 (EtOH)	$C_{16}H_{14}N_2O_3$	80	$\geq$100
b	2-Cl	181–181.5 (EtOH)	$C_{16}H_{13}N_2O_3Cl$	77	$\geq$100
c	4-OCH$_3$	197.5–198 (EtOH)	$C_{17}H_{16}N_2O_4$	72	$\geq$100
d	3,4-(OCH$_3$)$_2$	223–224 (EtOH)	$C_{18}H_{18}N_2O_5$	75	$\geq$100

[a] All of the compounds in this table gave satisfactory analyses for C, H, and N ($\pm$0.4%).

Table II. Physical Constants and Antiaggregation Activities of 7-(Ethoxycarbonyl)-6,8-dimethyl-2-(substituted-phenyl)-1(2H)-phthalazinone (2)

2	R	mp, °C (recrystn solvent)	formula[a]	inhibn of AA-induced platelet aggregation: ED_{50}, μM
a	H	138–139.5 (EtOH–hexane)	$C_{19}H_{18}N_2O_3$	2–5
b	2-CH$_3$	101–103 (EtOH–hexane)	$C_{20}H_{20}N_2O_3$	5–10
c	3-CH$_3$	111–113 (EtOH–hexane)	$C_{20}H_{20}N_2O_3$	50–100
d	4-CH$_3$	99–100 (EtOH–hexane)	$C_{20}H_{20}N_2O_3$	25–50
e	2-F	116–117 (EtOH–hexane)	$C_{19}H_{17}N_2O_3F$	2–5
f	4-F	131–131.5 (MeOH)	$C_{19}H_{17}N_2O_3F$	$\geq$50
g	2-Cl	125–126 (EtOH–hexane)	$C_{19}H_{17}N_2O_3Cl$	2–5
h	3-OCH$_3$	109–110 (MeOH)	$C_{20}H_{20}N_2O_4$	$\geq$50
i	4-OCH$_3$	100–100.5 (MeOH)	$C_{20}H_{20}N_2O_4$	5–10
j	3,4-(OCH$_3$)$_2$	178–179 (MeOH)	$C_{21}H_{22}N_2O_5$	$\geq$50
k	3-Cl, 4-CH$_3$	154–155 (MeOH)	$C_{20}H_{19}N_2O_3Cl$	$\geq$50
l	4-CO$_2$Et	167–168 (EtOH)	$C_{22}H_{22}N_2O_5$	$\geq$50
m	4-CO$_2$H	254–256 (EtOH)	$C_{20}H_{18}N_2O_5$	$\geq$50
n	4-SO$_2$CH$_3$	201–203 (MeOH)	$C_{20}H_{20}N_2O_5S$	$\geq$50
o	4-NO$_2$	149–151 (EtOH)	$C_{19}H_{17}N_3O_5$	$\geq$50
p	4-NH$_2$	133–134 (AcOEt–hexane)	$C_{19}H_{19}N_3O_3$	2–5
q	4-NHCOCH$_3$	193–194 (EtOH–ether)	$C_{21}H_{21}N_3O_4$	$\geq$50
r	4-OH	161.5–162.5 (AcOEt–hexane)	$C_{19}H_{18}N_2O_4$	25–50

[a] All of the compounds in this table gave satisfactory analyses for C, H, and N ($\pm$0.4%).

compounds **5a** and **5b**. The structures of these compounds were determined, respectively, on the basis of their NMR spectra. As judged by the characteristic signals of their NMR spectra, each mixture seemed to consist of nearly equal amounts of the two isomers.

Next, the preparation of **2** was carried out according to the following route as depicted in Scheme II. Ishizumi et al.[6] reported that carboxylic acid was reduced successfully into alcohol in good yield with sodium borohydride through mixed carbonic–carboxylic acid anhydrides. According to their method, the benzoic acid **7**, readily accessible by partial hydrolysis of the Diels–Alder adduct, was treated with ethyl chloroformate in the presence of triethylamine to give a mixed anhydride. The anhydride was reduced without isolation with NaBH$_4$ to afford **6a** in 86% yield. Bromination of **6a** with N-bromosuccinimide in CCl$_4$ in the presence of benzoyl peroxide gave the bromo compound **8**. By treatment of **8** with sodium acetate, **8** was converted to the corresponding acetoxy product, which was hydrolyzed with alkali to give **5a** in 81.6% yield. Reflux of **5a** with phenylhydrazine derivative in EtOH for 3–5 h afforded the final products **2** in good yield. The results are listed in Table II.

Several compounds **2o–r** were prepared from **2a** as shown in Scheme III. Nitration of **2a** with excess KNO$_3$ in concentrated H$_2$SO$_4$ gave trinitro compound **9** in 80% yield. With use of a 1.1 equimolar amount of KNO$_3$, a mononitro group was introduced selectively into the para position of the phenyl group of **2a** to provide **2o**. Hydrogenation of **2o** over 10% palladium charcoal afforded the amino derivative **2p** and the following acetylation gave the amido compound **2q** in the usual way. Diazotization of **2p** and subsequent heating of the resulting diazonium salt afforded the expected p-hydroxy derivative **2r**.

Finally, we synthesized 3,4-dihydrophthalazinone derivative from **2a** in order to study the influence of the presence of the 3,4-double bond on biological activity. Reduction of **2a** with zinc and acetic acid gave dihydro compound **10** in 52% yield accompanied with the formation of dehydroisoindole derivative **11** and acetanilide as shown in Scheme III. The formation of **11** should be due to the same ring contraction with N–N bond fission, which was reported to occur during reduction of phthalazone to phthalimidine by the treatment with zinc and hydrochloric acid.[7,8]

(6) Ishizumi, K.; Koga, K.; Yamada, S. *Chem. Pharm. Bull.* **1968**, *16*, 492.

(7) Daube, A. *Chem. Ber.* **1905**, *38*, 206.

Scheme III
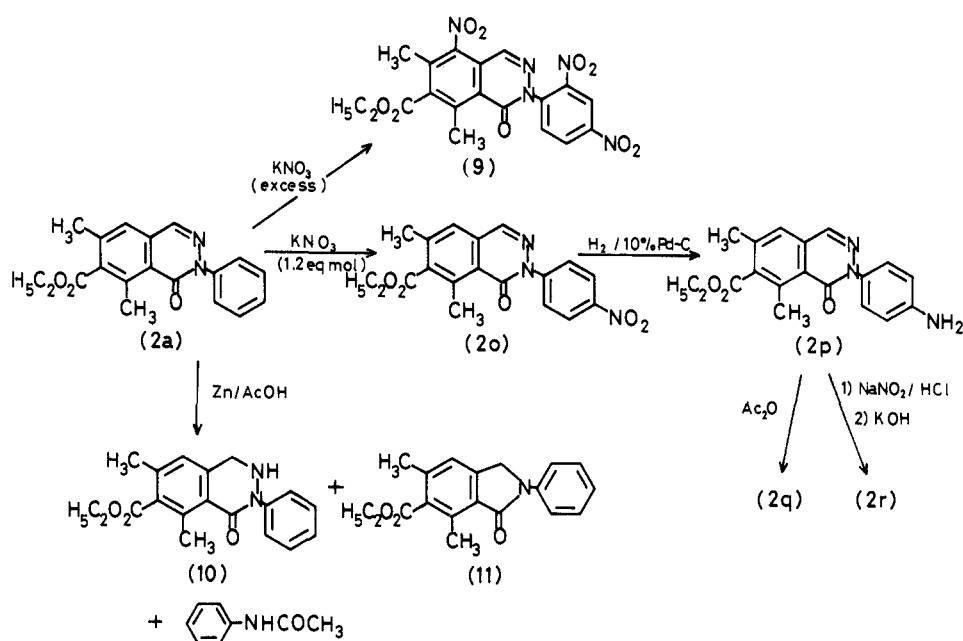
Biological Activity and Discussion

The inhibitory activities on platelet aggregation were assessed by the optical density method of Born,[9] using rabbit platelet rich plasma (PRP). Aggregation was induced by either adenosine diphosphate (ADP, 10 μM) or arachidonic acid (AA, 137 μM). In all test compounds, the dose–inhibition curves for AA-induced aggregation showed a steep pattern because the dose ranges between ED_0 (the highest dose without effect) and ED_{100} (the lowest dose which causes complete inhibition) were narrow. Therefore, the inhibitory activities are expressed terms of the range of ED_{50}. Althouth the ED_{50} values of 6,7-dimethoxy-2-phenyl-1(2H)-phthalazinone derivatives (3) were estimated to be 100 μM or more as shown in Table I, most of the compounds listed in Table II showed more potent inhibitory activity on the platelet aggregation induced by AA. The antiaggregating activities of 2a, 2e, 2g, and 2p were the most potent of all compounds tested herein.

As to the ADP-induced aggregation, all compounds shown in Table I did not produce any effect in a concentration of 100 μM. In contrast, some compounds such as 2a, 2e, 2g, and 2p in a concentration of 10 μM accelarated deaggregation. Representative tracings of the effect of 2a are illustrated in Figure 1. The other compounds (in concentrations of 50–100 μM) listed in Table II produced slight inhibition and acceleration of deaggregation (data not shown).

By comparison of the inhibitory activities of the positional isomers on phenyl group at the 2-position for AA-induced aggregation, it was found that the potency was decreased in the order of ortho > para > meta. This tendency is similar to the case of corresponding 4-hydroxymethyl derivatives as described earlier.[1] Concerning the kind of substituent on the phenyl group at the 2-position, electron-withdrawing groups at the para position such as in 2l, 2m, 2n, and 2o markedly reduced the potency, whereas electron-donating groups at the para position such as in 2d, 2i, and 2p relatively retained activity of the parent compound 2a. In contrast to para and meta substituents, the compound having a fluorine (2e)

(8) Darapsky, A.; Heinrichs, P. *J. Prakt. Chem.* 1936, *146*, 307.
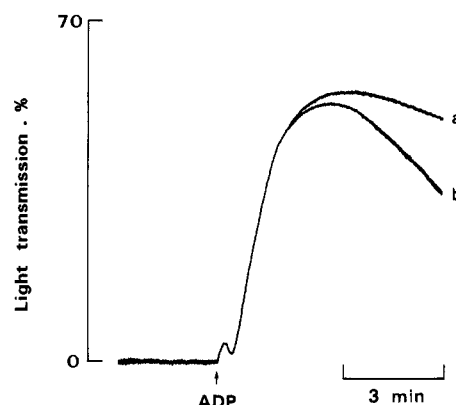(9) Born, G. V. R. *Nature (London)* 1962, *194*, 927.

Figure 1. Representative recordings of acceleration of deaggregation of ADP-induced aggregation by 2a. ADP was used in a final concentration of 10 μM. Results are representative of three separate experiments. (a) control, (b) 10 μM of 2a (see text).

or a chlorine atom (2g) or a methyl group (2b) at the ortho position constitutes the most active group in the series of the 2-phenyl-1(2H)-phthalazinone derivatives. However, inhibitory activities of these ortho-substituted compounds did not exceed that of nonsubstituted compound 2a, suggesting that the restriction of free rotation of phenyl group at the 2-position resulted in little increase in its pharmacological effect. Hydrogenation of the 3,4-double bond of the phthalazinone ring resulted in a complete loss of activity.

Pharmacological profiles of 2a and 2g were further investigated with respect to their mechanisms of antiaggregating action. Figure 2 shows the representative radiochromatogram of PG metabolites produced by incubation of [1-^{14}C]AA with rabbit washed platelet suspension (WPS) in the presence or absence of 2a. As shown in Figure 2, 2a in a concentration of 10 μM markedly inhibited the formation of thromboxane B_2 (TxB_2), a metabolite of TxA_2, and 12-hydroxy-5,8,10-heptadecatrienoic acid (HHT), one of the metabolites of PG endoperoxide, while 2a did not show any effect on the production of 12-hydroxy-5,8,10,14-eicosatetraenoic acid (HETE). Qualitatively similar results were obtained in the case of 10 μM of 2g (data not shown). In addition, the platelet aggre-
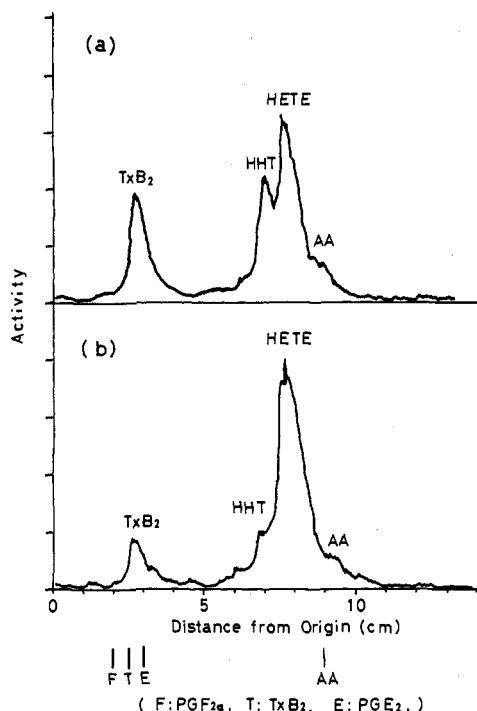
Figure 2. Radiochromatographical determination of the inhibitory effect of **2a** on production of TxB_2 and HHT from [1-^{14}C]AA by incubating with WPS. Authentic $PGF_{2\alpha}$, TxB_2, PGE_2, and AA were used for the identificaiton of each peak. (a) control, (b) 10 μM of **2a** (see text).
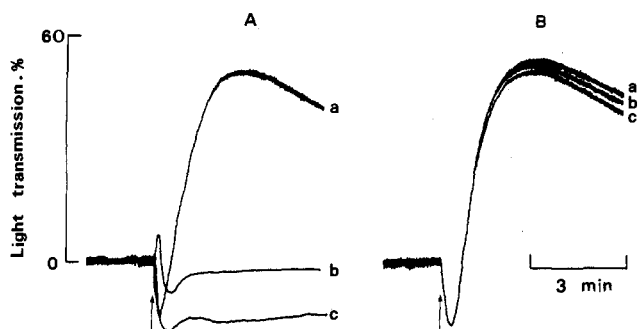

Figure 3. (A) Aggregation profile in rabbit PRP by an addition of PGH_2 and PM alone or in combination. (B) Effects of **2a** and **2g** on platelet aggregation induced by combined application of PGH_2 with PM. A: (a) PGH_2 + PM, (b) PGH_2 alone, (c) PM alone. B: (a) control, (b) 10 μM of **2a**, (c) 10 μM of indomethacin. Arrows indicate addition of PGH_2 and PM alone or in combination. Results are given as representative recordings of four separate determinations.

gation induced by the combined application of PGH_2 and platelet microsomes was unaffected by **2a** and **2g** in a concentration of 10 μM or by 10 μM of indomethacin (Figure 3), suggesting that the conversion of PGH_2 to TxA_2 is unaffected by **2a** and **2g** and that these two compounds might not antagonize TxA_2 activity. Thus, it seems likely that **2a** and **2g** inhibit AA-induced platelet aggregation mainly through the inhibition of cyclooxygenase activity and that the lipoxygenase pathway of arachidonate metabolism is unaffected by **2a** and **2g**, although the possibility that **2a** and **2g** may inhibit prostaglandin hydroperoxidase could not be denied. The biochemical mechanism of **2a** and **2g** is assumed to be similar to these of nonsteroidal antiinflammatory agents, such as aspirin and indomethacin, in this respect.

By inspection of CPK molecular models, it was found that the molecular shape of **2** was similar to that of papaverine. We examined, therefore, whether or not **2a** and

Table III. Inhibitory Effects of **2a**, **2g**, and Papaverine on cAMP Phosphodiesterase from Human Platelets

compd	IC$_{50}$, μM
2a	10
2g	34
papaverine	0.55

2g possess inhibitory action on cAMP phosphodiesterase, since papaverine is currently considered to inhibit platelet aggregation through inhibition of cAMP phosphodiesterase, thereby elevating the cellular level of cyclic AMP.[2] Table III shows the effects of **2a** and **2g** on cyclic AMP phosphodiesterase from human platelets in comparison with that of papaverine. The IC$_{50}$ value (the concentration that inhibits the enzyme activity by 50%) of papaverine was calculated to be 0.55 μM. In the cases of **2a** and **2g**, approximately 20 and 60 times higher concentrations, respectively, than that of papaverine were required for inhibition of the enzyme activity by 50%.

Though our intention to find papaverine-like biological activities in the phthalazinone derivatives failed, we have found phthalazinone derivatives such as **2a**, **2e**, **2g**, and **2p** having very potent inhibitory action on platelet aggregation. The detailed pharmacological studies on these compounds will be reported in a subsequent paper.

Experimental Section

Chemistry. all melting points were determined in a capillary tube and are uncorrected. IR spectra were determined with a Hitachi Model 285 spectrometer, UV spectra with a Hitachi Model 323 spectrometer, and NMR spectra with JEOL JUM-C-60HL spectrometer. Mass spectra (MS) were recorded with a Hitachi RMU-7L spectrometer; in all cases, direct sample insertion was carried out into the ion source.

General Procedure for the Preparation of 3. 2-(2-Chlorophenyl)-6,7-dimethoxy-1(2H)-phthalazinone (3b). A stirred mixture of 2-formyl-4,5-dimethoxybenzoic acid (0.5 g, 2.4 mmol), (o-chlorophenyl)hydrazine hydrochloride (0.43 g, 2.4 mmol), and EtOH (50 mL) was heated at 70 °C for 6 h under a nitrogen atmosphere. The mixture was concentrated under reduced pressure and then allowed to cool at room temperature. The resulting precipitate was filtered off and recrystallized from EtOH to give **3b** (0.58 g, 77%): mp 181-181.5 °C; MS, m/e 316 (M$^+$), 281, 265, 237; NMR (CDCl$_3$) δ 4.04 (6 H, s), 7.07 (1 H, s), 7.43 (4 H, s), 7.83 (1 H, s), 8.15 (1 H, s).

Reduction of 4-(Ethoxycarbonyl)-3,5-dimethylphthalic Anhydride (4) with LiAlH(OBu-t)$_3$. A mixture of LiAlH-(OBu-t)$_3$ (1 g, 3.54 mmol) in dry THF (30 mL) was added to a solution of anhydride 4 (747 mg, 3 mmol) in dry THF (30 mL) with stirring at room temperature. After stirring of the mixture for an additional 3 h, the solution was quenched with 1 N HCl solution (5 mL) and concentrated in vacuo. The residue was extracted with AcOEt and washed with brine. The organic layer was dried over Na$_2$SO$_4$ and concentrated. The residue was subjected to column chromatography on silica gel with 5% AcOEt-benzene (v/v, %). At first, an oily mixture of **6a** and **6b** was eluted. The TLC, IR, MS, and NMR data for the mixture were nearly identical with those of **6a** prepared by the unambiguous synthetic route described below. The attempt to separate the mixture was not carried out further. Next, the mixture of **5a** and **5b** was obtained. This oily mixture was rechromatographed on silica gel with 2% AcOEt-benzene. The fraction first eluted was recrystallized from ether-hexane to give pure **5b**: mp 121-122 °C; MS, m/e 250 (M$^+$), 205 (base ion peak); NMR (CDCl$_3$) δ 1.42 (3 H, t, J = 7 Hz), 2.35 (3 H, s), 2.39 (3 H, s), 4.45 (2 H, q, J = 7 Hz), 5.03 (1 H, d, J = 9 Hz disappeared by the addition of D$_2$O), 6.56 (1 H, d, J = 9 Hz singlet by the addition of D$_2$O), 7.43 (1 H, s). Anal. (C$_{13}$H$_{14}$O$_5$) C, H. The second eluted fraction was **5a**: mp 107-108.5 °C (ether-hexane); MS, m/e 250 (M$^+$), 205 (base ion peak); NMR (CDL$_3$) δ 1.42 (3 H, t, J = 7 Hz), 2.42 (3 H, s), 2.57 (3 H, s), 4.47 (2 H, q, J = 7 Hz), 5.00 (1 H, br s disappeared by the addition of D$_2$O), 6.55 (1 H, br s), 7.37 (1 H, s). Anal. (C$_{13}$H$_{14}$O$_5$) C, H.

6-(Ethoxycarbonyl)-5,7-dimethylphthalide (6a). A solution of the benzoic acid 7[10] (5.6 g, 20 mmol) and triethylamine (2.02 g, 20 mmol) in THF (40 mL) was treated dropwise with ethyl chloroformate (2.17 g, 20 mmol) in THF (10 mL) with stirring, while the temperature was maintained at –5 to 0 °C. After stirring for an additional 30 min at 0 °C, the precipitate was filtered off and washed with THF. The filtrate was added dropwise to a solution of NaBH$_4$ (1.89 g, 50 mmol) in 50% H$_2$O–THF (v/v, %) (40 mL), while the internal temperature was maintained below 15 °C. After the addition was completed, the mixture was allowed to stir at room temperature for 3 h. The mixture was acidified with dilute HCl and concentrated under reduced pressure. The residue was extracted with ether. The solvent was dried over Na$_2$SO$_4$ and evaporated. The residue was purified by column chromatography on silica gel with CHCl$_3$ to give 6a (4.02 g, 86%): mp 62–62.5 °C (ether–hexane); MS, m/e 234 (M$^+$), 206, 205, 189; NMR (CDCl$_3$) δ 1.44 (3 H, t, J = 7 Hz), 2.45 (3 H, s), 2.68 (3 H, s), 4.50 (2 H, q, J = 7 Hz), 5.27 (2 H, s), 7.23 (1 H, s). Anal. (C$_{13}$H$_{14}$O$_4$) C, H.

3-Bromo-6-(ethoxycarbonyl)-5,7-dimethylphthalide (8). A mixture of 6a (2.14 g, 9.15 mmol), N-bromosuccinimide (1.8 g, 10 mmol), a catalytic amount of benzoyl peroxide, and CCl$_4$ (50 mL) was refluxed for 4 h. The solvent was removed and the residue was chromatographed on a column of silica gel, eluted with benzene, to give 8 (1.99 g, 69.4%): mp 59–60 °C (benzene–hexane); MS m/e 269 and 267 (M$^+$ – OEt), 233 (base ion peak, M$^+$ – Br); NMR (CDCl$_3$) δ 1.42 (3 H, t, J = 7 Hz), 2.44 (3 H, s), 2.62 (3 H, s), 4.42 (2 H, q, J = 7 Hz), 7.26 (2 H, s). Anal. (C$_{13}$H$_{13}$O$_4$Br) C, H.

6-(Ethoxycarbonyl)-3-hydroxy-5,7-dimethylphthalide (5a). Sodium acetate (310 mg, 3.8 mmol) was added to 3-bromophthalide 8 (784 mg, 3.14 mmol) in AcOH (25 mL). The solution was heated at 70–80 °C for 1 h. After removal of excess AcOH in vacuo, aqueous 1 N KOH (12 mL) was added to the residue. The mixture was allowed to stand overnight at room temperature. The solution was acidified with dilute HCl and extracted with AcOEt. The organic layer was dried over Na$_2$SO$_4$ and concentrated. The residue was recrystallized from ether–hexane to give 5a (642 mg, 81.6%): mp 107–108.5 °C. Spectral data of NMR and MS agreed with those of compound obtained by reduction of 4.

General Procedure for the Preparation of 2. 7-(Ethoxycarbonyl)-6,8-dimethyl-2-phenyl-1(2H)-phthalazinone (2a). A solution of 5a (2.5 g, 10 mmol) and phenylhdrazine (1.2 g, 11 mmol) in EtOH (150 mL) was refluxed for 3 h. The solvent was concentrated under reduced pressure. After the addition of H$_2$O, the mixture was extracted with AcOEt. The organic layer was washed with dilute HCl and H$_2$O, dried over MgSO$_4$, and concentrated. The product was recrystallized from EtOH–hexane to give 2a (2.1 g, 65%): mp 138–139.5 °C MS, m/e 322 (M$^+$); NMR (CDCl$_3$) δ 1.43 (3 H, t, J = 7 Hz), 2.47 (3 H, s), 2.89 (3 H, s), 4.48 (2 H, q, J = 7 Hz), 7.30–7.80 (6 H, m), 8.13 (1 H, s); IR ν$_{max}$^{KBr} 1723, 1670 cm^{-1}; UV λ$_{max}$^{EtOH} 2.28 nm (log ε 4.67), 322 (4.33).

2-(2-Chlorophenyl)-7-(ethoxycarbonyl)-6,8-dimethyl-1-(2H)-phthalazinone (2g). A mixture of 5a (555 mg, 2.2 mmol), (o-chlorophenyl)hydrazine hydrochloride (437 mg, 2.42 mmol), NaOAc (200 mg, 2.42 mmol), and EtOH (20 mL) was refluxed for 20 h. The solvent was concentrated, treated with 10% HCl solution, and extracted with AcOEt. The organic layer was dried over MgSO$_4$ and concentrated. The residue was column chromatographed on silica gel, eluted with 1% AcOEt in benzene (v/v, %) to afford 2g (459 mg, 58%): mp 125–126 °C (EtOH–hexane); MS, m/e 356 (M$^+$), 321; NMR (CDCl$_3$) δ 1.43 (3 H, t, J = 7 Hz), 2.47 (3 H, s), 2.88 (3 H, s), 4.47 (2 H, q, J = 7 Hz), 7.25–7.70 (5 H, m), 8.12 (1 H, s); IR ν$_{max}$^{KBr} 1738, 1660 cm^{-1}; UV λ$_{max}$^{EtOH} 217 nm (log ε 4.64), 30.9 (4.05).

7-(Ethoxycarbonyl)-6,8-dimethyl-5-nitro-2-(2,4-dinitrophenyl)-1(2H)-phthalazinone (9). A stirred solution of 2a (2.26 g, 7.01 mmol) in 95% H$_2$SO$_4$ (30 mL) was treated portionwise with KNO$_3$ (2.2 g, 21.76 mmol) at 0 °C. The mixture was allowed to stir overnight at room temperature and then poured onto ice and extracted with CHCl$_3$. The CHCl$_3$ extract was washed with

saturated aqueous NaHCO$_3$, dried over Na$_2$SO$_4$, and concentrated. The residue was recrystallized from benzene–EtOH to give 9 (2.56 g, 79.8%): mp 115–119 °C; MS, m/e 457 (M$^+$), 412; NMR (CDCl$_3$) δ 1.45 (3 H, t, J = 7 Hz), 2.43 (3 H, s), 2.83 (3 H, s), 4.50 (2 H, q, J = 7 Hz), 7.91 (1 H, d, J = 9 Hz), 8.62 (1 H, dd, J = 9, 3 Hz), 9.96 (1 H, d, J = 3 Hz). Anal. (C$_{19}$H$_{15}$N$_5$O$_9$) C, H, N.

7-(Ethoxycarbonyl)-6,8-dimethyl-2-(4-nitrophenyl)-1-(2H)-phthalazinone (2o). A portion (2.01 g, 6.24 mmol) of 2a was dissolved in 95% H$_2$SO$_4$ (30 mL). Potassium nitrate (725 mg, 7.2 mmol) was added portionwise to the solution, while the temperature was maintained at –5 to 5 °C. After stirring for an additional 5 h at 0 °C, the mixture was poured onto ice and extracted with CHCl$_3$. The extract was washed with saturated aqueous NaHCO$_3$ solution, dried over Na$_2$SO$_4$, and concentrated. The residue was recrystallized from EtOH to give 2o (1.15 g, 50%): mp 149–151 °C; MS, m/e 367 (M$^+$), 338, 322; NMR (CDCl$_3$) δ 1.45 (3 H, t, J = 7 Hz), 2.50 (3 H, s), 2.90 (3 H, s), 4.54 (2 H, q, J = 7 Hz), 7.49 (1 H, s), 7.95 (2 H, d, J = 10 Hz), 8.23 (1 H, s), 8.42 (2 H, d, J = 10 Hz); IR ν$_{max}$^{KBr} 1735, 1520, 1350 cm^{-1}.

2-(4-Aminophenyl)-7-(ethoxycarbonyl)-6,8-dimethyl-1-(2H)-phthalazinone (2p). The nitro compound 2o (2.9 g, 7.8 mmol) was dissolved in 50 mL of hot dioxane and hydrogenated over 10% Pd–C (340 mg) at atmospheric pressure at room temperature. When absorption of the theoretical amount of H$_2$ was complete, the catalyst was filtered and the solvent was evaporated. The residue was recrystallized from AcOEt–hexane to give 2p (2.31 g, 86.7%): mp 133–134 °C, MS, m/e 337 (M$^+$); NMR (CDCl$_3$) δ 1.42 (3 H, t, J = 7 Hz), 2.43 (3 H, s), 2.89 (3 H, s), 3.22 (2 H br s, disappeared by the addition of D$_2$O), 4.46 (2 H, q, J = 7 Hz), 6.71 (2 H, d, J = 9 Hz), 7.33 (2 H, d, J = 9 Hz), 7.36 (1 H, s), 8.08 (1 H, s).

2-[4-(Acetylamino)phenyl]-7-(ethoxycarbonyl)-6,8-dimethyl-1(2H)-phthalazinone (2q). The compound 2p was acetylated at room temperature with acetic anhydride in pyridine by a usual procedure. Conventional treatment of the reaction mixture and recrystallization from EtOH–ether gave 2q in nearly quantitative yield: mp 193–194 °C; MS, m/e 379 (M$^+$); NMR (CDCl$_3$) δ 1.44 (3 H, t, J = 7 Hz), 2.13 (3 H, s), 2.42 (3 H, s), 2.89 (3 H, s), 4.49 (2 H, q, J = 7 Hz), 7.40 (1 H, s), 8.05 (1 H, br s, disappeared by the addition of D$_2$O), 8.12 (1 H, s).

7-(Ethoxycarbonyl)-2-(4-hydroxyphenyl)-6,8-dimethyl-1-(2H)-phthalazinone (2r). A stirred solution of 2p (989 mg, 2.93 mmol) in THF (5 mL) was treated with 1 N HCl solution (15 mL) and was added dropwise with NaNO$_2$ (243 mg, 3.51 mmol) in H$_2$O (3 mL) with ice cooling. The mixture was stirred for an additional 30 min at 0 °C, subsequently for 30 min at room temperature, and for 2 h at 60–70 °C. The mixture was extracted with benzene. The benzene layer was extracted with 1 N KOH solution. The aqueous phase was acidified with HCl and extracted with benzene. The benzene fraction was dried over Na$_2$SO$_4$ and concentrated. The product was recrystallized from AcOEt–hexane to give 2r (163 mg, 16.4%): mp 161.5–162.5 °C; MS m/e 338 (M$^+$), 309; NMR (CDCl$_3$) δ 1.44 (3 H, t, J = 7 Hz), 2.47 (3 H, s), 2.84 (3 H, s), 4.48 (2 H, q, J = 7 Hz), 6.68 (2 H, d, J = 9 Hz), 7.23 (1 H, s, disappeared by the addition of D$_2$O), 7.27 (1 H, s), 7.30 (2 H, d, J = 9 Hz), 8.12 (1 H, s).

Reduction of 7-(Ethoxycarbonyl)-6,8-dimethyl-2-phenyl-1(2H)-phthalazinone (2a) with Zn/AcOH. A portion (1.07 g, 3.32 mmol) of 2a was dissolved in 30 mL of AcOH containing 1 mL of H$_2$O. Activated Zn dust (3 g)[11] was added to the solution. The mixture was refluxed overnight and then diluted with H$_2$O. The solution was made alkaline with NaHCO$_3$ and extracted with AcOEt. The AcOEt layer was dried over Na$_2$SO$_4$ and concentrated. The products were separated by column chromatography on silica gel with CHCl$_3$. The following products were obtained in the order of elution.

10: mp 156–158 °C (CHCl$_3$–ether) (563 mg, 52.3%); MS m/e 327 (M$^+$), 279, 218; NMR (CDCl$_3$) δ 1.41 (3 H, t, J = 7 Hz), 2.33 (3 H, s), 2.65 (3 H, s), 4.17 (2 H, d, J = 7 Hz, singlet by the addition of D$_2$O), 4.43 (2 H, q, J = 7 Hz), 4.75 (1 H, br t, J = 7 Hz, disappeared by the addition of D$_2$O), 6.80 (1 H, s), 7.10–7.90 (5 H, m). Anal. (C$_{19}$H$_{20}$N$_2$O$_3$) C, H, N.

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Acetanilide: 101 mg, 22.5%.

11: mp 206–207 °C (CHCl$_3$–ether) (169 mg, 21.8%); MS, m/e 233 (M$^+$), 204, 188, 160; NMR (CDCl$_3$) δ 1.41 (3 H, t, J = 7 Hz), 2.39 (3 H, s), 2.70 (3 H, s), 4.37 (2 H, s), 4.43 (2 H, q, J = 7 Hz), 7.12 (1 H, s), 7.42 (1 H, br s, disappeared on heating in the presence of D$_2$O); IR ν_{max}^{KBr} 1735, 1695 cm^{-1}. Anal. (C$_{19}$H$_{19}$NO$_3$) C, H, N.

Biological Activity. Preparation of Platelet-Rich Plasma (PRP). Blood samples were collected into tube containing one-tenth volume of 3.8% aqueous sodium citrate through a cannula inserted into the carotid artery of rabbits anesthetized with sodium pentobarbital (35 mg/kg, iv). PRP was prepared by centrifugation of the blood samples for 15 min at 150g at room temperature and platelet-poor plasma (PPP) was obtained by further centrifugation of the blood at 1670g for 15 min. The aggregation induced by AA or ADP appears to be practically unaffected by the anesthetic.

Platelet Aggregation Test. The turbidometric method of Born[9] modified to provide continuous stirring (1200 rpm) and maintenance of constant temperature (37 °C) was employed for assessing the ability of test compounds to inhibit platelet aggregation induced by aggregating agents. A 0.435-mL sample of PRP was placed in an aggregometer (SIENCO, dual sample aggregation meter, Model DP-247E) and then a various concentration of the test compound or vehicle in a volume of 2.5 μL was added. After preincubation of the mixture of PRP and test compound or vehicle for 3 min, 10 μL of an aqueous solution of ADP (final concentration of 10 μM) or 10 μL of an aqueous solution of AA (final concentration of 137 μM) was added to induce platelet aggregation. Inhibition of platelet aggregation by a test compound was calculated by dividing the maximum deflection in the optical density curve by that without test compound. Test compounds were dissolved in dimethyl sulfoxide (Me$_2$SO), which was present in a final concentration of 0.5% or less in all experiments and had no effect at this concentration on any parameters studied. The number of platelets (ranging from 3.5 × 10^5 to 4.5 × 10^5 platelets/μL of PRP) was determined with the aid of a Coulter Counter (Coulter Electronics, Inc.). Results are given as the range of ED$_{50}$ values on three separate experiments.

Thromboxane A$_2$ Induced Platelet Aggregation. Two micrograms of prostaglandin H$_2$ (PGH$_2$), which had been prepared by the method of Yoshimoto et al.,[12] was dried under a stream of N$_2$ in a cuvette. Then, the bovine platelet microsomes (BPM, 0.8 mg of protein) suspended in 500 μL of 50 mM Tris buffer (pH 7.8) were added to the cuvette. The mixture of PGH$_2$ and BPM was then incubated at 0 °C for 2 min in the presence or absence of test agents. Magnitude of aggregation was examined when an 80-μL aliquot of the incubation mixture was added into a cuvette containing 0.420 μL of PRP.

Radiochemical Experiment Using Washed Platelet Suspension (WPS). WPS was prepared by the method of Svensson et al.[13] WPA (2 mL, 1 × 10^6 platelets/1 μL) was preincubated with a solution of each test compound or Me$_2$SO (control) and then incubated with [1-^{14}C]arachidonic acid (2 μCi in a final concentration of 137 μM AA) at 37 °C for 20 min. The reaction mixture was diluted with H$_2$O, and 5 volumes with MeOH were added. The mixture was acidified to pH 3.5 with 1 N HCl and extracted twice with AcOEt. The organic layer was washed with H$_2$O and dried over Na$_2$SO$_4$. After evaporation of the solvent, the residue was dissolved in ether (2 mL) and a 100-μL aliquot was spotted on silica gel plate (60 F$_{254}$ glass plate for thin-layer chromatography; E. Merck). The thin-layer chromatography was carried out in the following solvent system: isooctane–AcOEt–AcOH–H$_2$O, 5:11:2:10, v/v. Authentic TxB$_2$, AA, PGE$_2$, and PGF$_{2\alpha}$ were also developed on the plate, and the spots on the plate were visualized by exposure to iodine vapor, and radioactivity was assessed by means of radiochromato scanner (Packard Model 7220 scanner).

Assay of cAMP Phosphodiesterase (PDE). Preparation of the low K_m cAMP PDE and assay of the enzyme activity were carried out according to the method previously described.[14] The inhibitory activity of the test agents is given in terms of IC$_{50}$ (μM concentration that inhibits enzyme activity by 50%).

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Registry No. 2a, 75357-55-0; 2b, 73726-24-6; 2c, 73726-16-6; 2d, 73726-15-5; 2e, 91110-32-6; 2f, 73726-07-5; 2g, 73726-05-3; 2h, 73726-14-4; 2i, 73726-12-2; 2j, 73726-19-9; 2k, 73726-18-8; 2l, 73726-32-6; 2m, 73726-21-3; 2n, 91110-33-7; 2o, 73731-49-4; 2p, 73726-22-4; 2q, 91110-34-8; 2r, 73726-23-5; 3a, 79285-40-8; 3b, 78865-49-3; 3c, 78865-56-2; 3d, 78865-57-3; 4, 56611-62-2; 5a, 73726-04-2; 5b, 91110-31-5; 6a, 77473-58-6; 6b, 91110-30-4; 7, 56863-79-7; 8, 77473-59-7; 9, 91110-35-9; 10, 91110-36-0; 11, 91110-37-1; H$_2$NNHC$_6$H$_5$, 100-63-0; H$_2$NNHC$_6$H$_4$-o-Me, 529-27-1; H$_2$NNHC$_6$H$_4$-m-Me, 536-89-0; H$_2$NNHC$_6$H$_4$-p-Me, 539-44-6; H$_2$NNHC$_6$H$_4$-o-F, 2368-80-1; H$_2$NNHC$_6$H$_4$-p-F, 371-14-2; H$_2$NNHC$_6$H$_4$-o-Cl·HCl, 41052-75-9; H$_2$NNHC$_6$H$_4$-m-OMe, 15384-39-1; H$_2$NNHC$_6$H$_4$-p-OMe, 3471-32-7; 3,4-(OCH$_3$)$_2$C$_6$H$_3$NHNH$_2$, 63756-98-9; 3-Cl,4-MeC$_6$H$_4$NHNH$_2$, 51304-65-5; H$_2$NNHC$_6$H$_4$-p-CO$_2$Et, 14685-90-6; H$_2$NNHC$_6$H$_4$-p-CO$_2$H, 619-67-0; H$_2$NNHC$_6$H$_4$-p-SO$_2$CH$_3$, 877-66-7; H$_2$NNHC$_6$H$_5$·HCl, 59-88-1; H$_2$NNHC$_6$H$_4$-p-O-Me·HCl, 19501-58-7; 3,4-(OCH$_3$)$_2$-C$_6$H$_3$-NH-NH$_2$·HCl, 40119-17-3; 2-formyl-4,5-dimethoxybenzoic acid, 490-63-1; acetanilide, 103-84-4.

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