

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

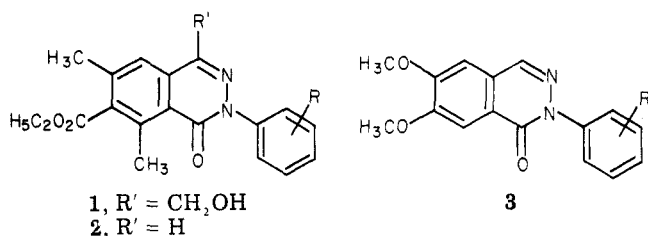
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7-(Ethoxycarbonyl)-6,8-dimethyl-2-phenyl-1(2H)-phthalazinone derivatives and several analogues were synthesized and their inhibitory effects on platelet aggregation were evaluated. Structure-activity relationships are discussed. All synthesized compounds 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 earlier¹ the synthesis of 7-(ethoxycarbonyl)-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-(ethoxycarbonyl)-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.

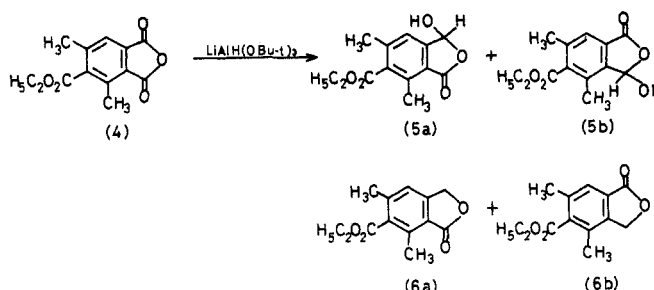
We report herein the syntheses of 6,7-dimethoxy-2-(substituted-phenyl)-1(2H)-phthalazinone (3) and 7-(ethoxycarbonyl)-6,8-dimethyl-2-(substituted-phenyl)-1(2H)-phthalazinone derivatives (2) and the structure-activity relationship of these compounds with respect to antiaggregating activity.



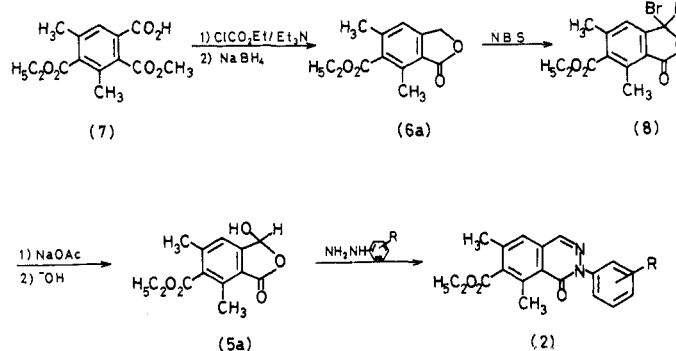
Chemistry. The synthesis of 6,7-dimethoxy-1(2H)-phthalazinone (3a) through the reaction of 2-formyl-4,5-dimethoxybenzoic acid (*m*-opianic acid) with phenylhydrazine had been reported by Fargher et al.³ According to their method, the derivatives having a substituent on the 2-phenyl group of 3a were prepared, 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 μ M. 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

Scheme I



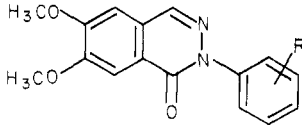
Scheme II



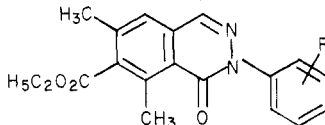
examined. It was assumed that the best intermediate to prepare the derivatives of 2 bearing a substituent on the 2-phenyl group was 6-(ethoxycarbonyl)-5,7-dimethyl-3-hydroxyphthalide (5a). The reduction of substituted phthalic anhydrides with various agents to the corresponding 3-hydroxyphthalides was 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 regioselectivity. 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

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- (1) Sugimoto, A.; Ito, S.; Eguchi, Y.; Tanaka, H.; Takashima, Y.; Ishikawa, M. *Eur. J. Med. Chem.*, submitted for publication.
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- (3) Fargher, R. G.; Perkin, W. H., Jr. *J. Chem. Soc.* 1921, 1724.
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Table I. Physical Constants and Antiaggregation Activities of 6,7-Dimethoxy-2-(substituted-phenyl)-1(2*H*)-phthalazinone (3)


3	R	mp, °C (recrystn solvent)	formula ^a	yield, %	inhibn of AA-induced platelet aggre- gation: ED ₅₀ , μM
a	H	227–228 (EtOH)	C ₁₆ H ₁₄ N ₂ O ₃	80	≥100
b	2-Cl	181–181.5 (EtOH)	C ₁₆ H ₁₃ N ₂ O ₃ Cl	77	≥100
c	4-OCH ₃	197.5–198 (EtOH)	C ₁₇ H ₁₆ N ₂ O ₄	72	≥100
d	3,4-(OCH ₃) ₂	223–224 (EtOH)	C ₁₈ H ₁₈ N ₂ O ₅	75	≥100

^a All of the compounds in this table gave satisfactory analyses for C, H, and N (±0.4%).**Table II.** Physical Constants and Antiaggregation Activities of 7-(Ethoxycarbonyl)-6,8-dimethyl-2-(substituted-phenyl)-1(2*H*)-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 ₂ O ₃	2–5
b	2-CH ₃	101–103 (EtOH–hexane)	C ₂₀ H ₂₀ N ₂ O ₃	5–10
c	3-CH ₃	111–113 (EtOH–hexane)	C ₂₀ H ₂₀ N ₂ O ₃	50–100
d	4-CH ₃	99–100 (EtOH–hexane)	C ₂₀ H ₂₀ N ₂ O ₃	25–50
e	2-F	116–117 (EtOH–hexane)	C ₁₉ H ₁₇ N ₂ O ₃ F	2–5
f	4-F	131–131.5 (MeOH)	C ₁₉ H ₁₇ N ₂ O ₃ F	≥50
g	2-Cl	125–126 (EtOH–hexane)	C ₁₉ H ₁₇ N ₂ O ₃ Cl	2–5
h	3-OCH ₃	109–110 (MeOH)	C ₂₀ H ₂₀ N ₂ O ₄	≥50
i	4-OCH ₃	100–100.5 (MeOH)	C ₂₀ H ₂₀ N ₂ O ₄	5–10
j	3,4-(OCH ₃) ₂	178–179 (MeOH)	C ₂₁ H ₂₂ N ₂ O ₅	≥50
k	3-Cl, 4-CH ₃	154–155 (MeOH)	C ₂₀ H ₁₉ N ₂ O ₃ Cl	≥50
l	4-CO ₂ Et	167–168 (EtOH)	C ₂₂ H ₂₂ N ₂ O ₅	≥50
m	4-CO ₂ H	254–256 (EtOH)	C ₂₀ H ₁₈ N ₂ O ₅	≥50
n	4-SO ₂ CH ₃	201–203 (MeOH)	C ₂₀ H ₂₀ N ₂ O ₅ S	≥50
o	4-NO ₂	149–151 (EtOH)	C ₁₉ H ₁₇ N ₃ O ₅	≥50
p	4-NH ₂	133–134 (AcOEt–hexane)	C ₁₉ H ₁₉ N ₃ O ₃	2–5
q	4-NHCOCH ₃	193–194 (EtOH–ether)	C ₂₁ H ₂₁ N ₃ O ₄	≥50
r	4-OH	161.5–162.5 (AcOEt–hexane)	C ₁₉ H ₁₈ N ₂ O ₄	25–50

^a All of the compounds in this table gave satisfactory analyses for C, H, and N (±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 NaBH₄ to afford **6a** in 86% yield. Bromination of **6a** with *N*-bromosuccinimide in CCl₄ 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.

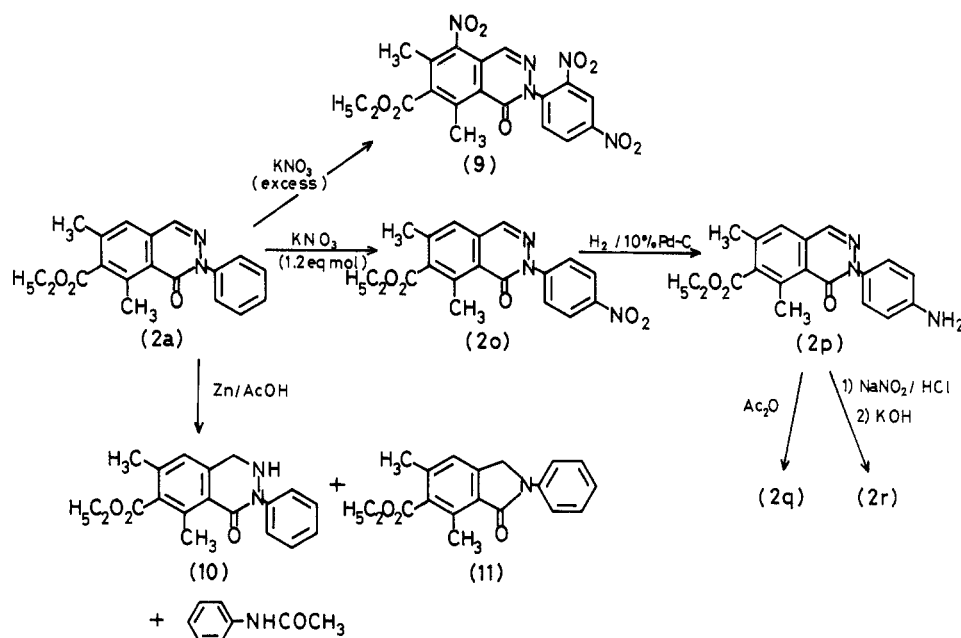
Several compounds **2o–r** were prepared from **2a** as shown in Scheme III. Nitration of **2a** with excess KNO₃ in concentrated H₂SO₄ gave trinitro compound **9** in 80% yield. With use of a 1.1 equimolar amount of KNO₃, a mononitro group was introduced selectively into the para position of the phenyl 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 dehydroisindole 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}

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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_{50} (the highest dose without effect) and ED_{100} (the lowest dose which causes complete inhibition) were narrow. Therefore, the inhibitory activities are expressed in terms of the range of ED_{50} . Although the ED_{50} values of 6,7-dimethoxy-2-phenyl-1(2*H*)-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 accelerated 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**)

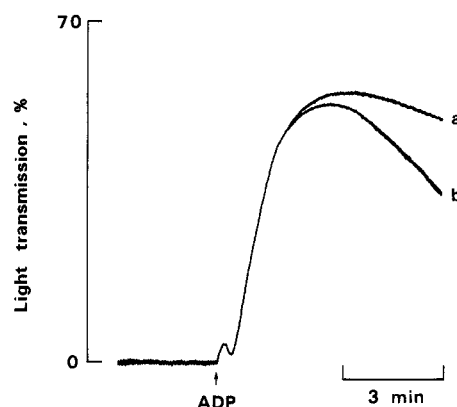


Figure 1. Representative recordings of acceleration of deaggregation of ADP-induced aggregation by **2a**. ADP was used in a final concentration of 10 μM . Results are representative of three separate experiments. (a) control, (b) 10 μM of **2a** (see text).

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(2*H*)-phthalazinone derivatives. However, inhibitory activities of these ortho-substituted compounds did not exceed that of unsubstituted 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 [$1\text{-}^{14}\text{C}$]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-

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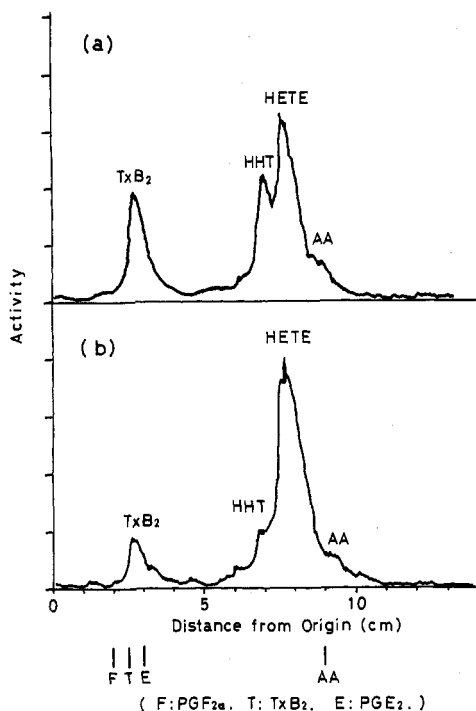


Figure 2. Radiochromatographical determination of the inhibitory effect of **2a** on production of TxB_2 and HHT from $[1\text{-}^{14}\text{C}]\text{AA}$ by incubating with WPS. Authentic $\text{PGF}_{2\alpha}$, TxB_2 , PGE_2 , and AA were used for the identification of each peak. (a) control, (b) $10\text{ }\mu\text{M}$ of **2a** (see text).

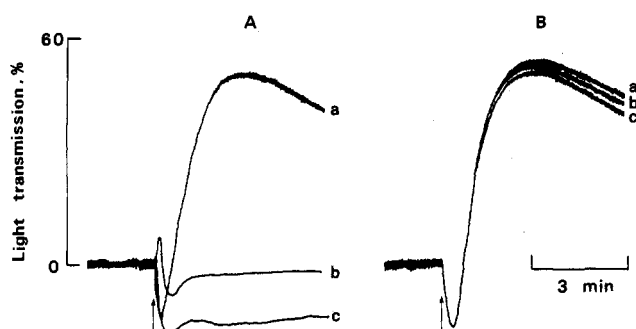


Figure 3. (A) Aggregation profile in rabbit PRP by an addition of PGH_2 and PM alone or in combination. (B) Effects of **2a** and **2g** on platelet aggregation induced by combined application of PGH_2 with PM. A: (a) PGH_2 + PM, (b) PGH_2 alone, (c) PM alone. B: (a) control, (b) $10\text{ }\mu\text{M}$ of **2a**, (c) $10\text{ }\mu\text{M}$ 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 $10\text{ }\mu\text{M}$ or by $10\text{ }\mu\text{M}$ 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 $0.55\text{ }\mu\text{M}$. 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(2*H*)-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 $70\text{ }^\circ\text{C}$ 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 $181\text{--}181.5\text{ }^\circ\text{C}$; 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 $\text{LiAlH}(\text{O}i\text{Bu})_3$. A mixture of $\text{LiAlH}(\text{O}i\text{Bu})_3$ (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_2SO_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 $121\text{--}122\text{ }^\circ\text{C}$; MS, m/e 250 (M^+), 205 (base ion peak); NMR (CDCl_3) δ 1.42 (3 H, t, $J = 7\text{ Hz}$), 2.35 (3 H, s), 2.39 (3 H, s), 4.45 (2 H, q, $J = 7\text{ Hz}$), 5.03 (1 H, d, $J = 9\text{ Hz}$ disappeared by the addition of D_2O), 6.56 (1 H, d, $J = 9\text{ Hz}$ singlet by the addition of D_2O), 7.43 (1 H, s). Anal. ($\text{C}_{13}\text{H}_{14}\text{O}_5$) C, H. The second eluted fraction was **5a**: mp $107\text{--}108.5\text{ }^\circ\text{C}$ (ether-hexane); MS, m/e 250 (M^+), 205 (base ion peak); NMR (CDCl_3) δ 1.42 (3 H, t, $J = 7\text{ Hz}$), 2.42 (3 H, s), 2.57 (3 H, s), 4.47 (2 H, q, $J = 7\text{ Hz}$), 5.00 (1 H, br s disappeared by the addition of D_2O), 6.55 (1 H, br s), 7.37 (1 H, s). Anal. ($\text{C}_{13}\text{H}_{14}\text{O}_5$) 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 NaBH₄ (1.89 g, 50 mmol) in 50% H₂O-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 Na₂SO₄ and evaporated. The residue was purified by column chromatography on silica gel with CHCl₃ to give **6a** (4.02 g, 86%): mp 62–62.5 °C (ether-hexane); MS, *m/e* 234 (M⁺), 206, 205, 189; NMR (CDCl₃) 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. (C₁₃H₁₄O₄) 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 CCl₄ (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 (CDCl₃) 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. (C₁₃H₁₃O₄Br) 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 Na₂SO₄ 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 phenylhydrazine (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 H₂O, the mixture was extracted with AcOEt. The organic layer was washed with dilute HCl and H₂O, dried over MgSO₄, 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 (CDCl₃) 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 $\lambda_{\max}^{\text{EtOH}}$ 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 MgSO₄ 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 (CDCl₃) 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 $\lambda_{\max}^{\text{EtOH}}$ 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% H₂SO₄ (30 mL) was treated portionwise with KNO₃ (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 CHCl₃. The CHCl₃ extract was washed with

saturated aqueous NaHCO₃, dried over Na₂SO₄, 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 (CDCl₃) 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. (C₁₉H₁₅N₅O₉) 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% H₂SO₄ (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 CHCl₃. The extract was washed with saturated aqueous NaHCO₃ solution, dried over Na₂SO₄, 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 (CDCl₃) 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 H₂ 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 (CDCl₃) 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 D₂O), 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 (CDCl₃) 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 (5 H, s), 8.05 (1 H, br s, disappeared by the addition of D₂O), 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 NaNO₂ (243 mg, 3.51 mmol) in H₂O (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 Na₂SO₄ 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 (CDCl₃) 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 D₂O), 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 H₂O. Activated Zn dust (3 g)¹¹ was added to the solution. The mixture was refluxed overnight and then diluted with H₂O. The solution was made alkaline with NaHCO₃ and extracted with AcOEt. The AcOEt layer was dried over Na₂SO₄ and concentrated. The products were separated by column chromatography on silica gel with CHCl₃. The following products were obtained in the order of elution.

10: mp 156–158 °C (CHCl₃-ether) (563 mg, 52.3%); MS *m/e* 327 (M⁺), 279, 218; NMR (CDCl₃) 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 D₂O), 4.43 (2 H, q, *J* = 7 Hz), 4.75 (1 H, br t, *J* = 7 Hz, disappeared by the addition of D₂O), 6.80 (1 H, s), 7.10–7.90 (5 H, m). Anal. (C₁₉H₂₀N₂O₃) C, H, N.

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Acetanilide: 101 mg, 22.5%.

11: mp 206–207 °C (CHCl₃-ether) (169 mg, 21.8%); MS, *m/e* 233 (M⁺), 204, 188, 160; NMR (CDCl₃) 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 D₂O); IR $\nu_{\text{max}}^{\text{KBr}}$ 1735, 1695 cm⁻¹. Anal. (C₁₉H₁₉NO₃) 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 Born⁹ 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 μ L was added. After preincubation of the mixture of PRP and test compound or vehicle for 3 min, 10 μ L of an aqueous solution of ADP (final concentration of 10 μ M) or 10 μ L of an aqueous solution of AA (final concentration of 137 μ M) 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 (Me₂SO), 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/ μ L of PRP) was determined with the aid of a Coulter Counter (Coulter Electronics, Inc.). Results are given as the range of ED₅₀ values on three separate experiments.

Thromboxane A₂ Induced Platelet Aggregation. Two micrograms of prostaglandin H₂ (PGH₂), which had been prepared by the method of Yoshimoto et al.,¹² was dried under a stream of N₂ in a cuvette. Then, the bovine platelet microsomes (BPM, 0.8 mg of protein) suspended in 500 μ L of 50 mM Tris buffer (pH 7.8) were added to the cuvette. The mixture of PGH₂ 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- μ L aliquot of the incubation mixture was added into a cuvette containing 0.420 μ L 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/ μ L) was preincubated with a solution of each test compound or Me₂SO (control) and then incubated with [1-¹⁴C]arachidonic acid (2 μ Ci in a final concentration of 137 μ M AA) at 37 °C for 20 min. The reaction mixture was diluted with H₂O, and 5 volumes of 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 H₂O and dried over Na₂SO₄. After evaporation of the solvent, the residue was dissolved in ether (2 mL) and a 100- μ L aliquot was spotted on silica gel plate (60 F₂₅₄ glass plate for thin-layer chromatography; E. Merck). The thin-layer chromatography was carried out in the following solvent system: isooctane-AcOEt-AcOH-H₂O, 5:11:2:10, v/v. Authentic TxB₂, AA, PGE₂, and PGF_{2 α} 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 IC₅₀ (μ M 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; H₂NNHC₆H₅, 100-63-0; H₂NNHC₆H₄-o-Me, 529-27-1; H₂NNHC₆H₄-m-Me, 536-89-0; H₂NNHC₆H₄-p-Me, 539-44-6; H₂NNHC₆H₄-o-F, 2368-80-1; H₂NNHC₆H₄-p-F, 371-14-2; H₂NNHC₆H₄-o-Cl·HCl, 41052-75-9; H₁NNHC₆H₄-m-OMe, 15384-39-1; H₂NNHC₆H₄-p-OMe, 3471-32-7; 3,4-(OCH₃)₂C₆H₃NHNH₂, 63756-98-9; 3-Cl,4-MeC₆H₄NHNH₂, 51304-65-5; H₂NNHC₆H₄-p-CO₂Et, 14685-90-6; H₂NNHC₆H₄-p-CO₂H, 619-67-0; H₁NNHC₆H₄-p-SO₂CH₃, 877-66-7; H₂NNHC₆H₅·HCl, 59-88-1; H₂NNHC₆H₄-p-O-Me·HCl, 19501-58-7; 3,4-(OCH₃)₂-C₆H₃-NH-NH₂·HCl, 40119-17-3; 2-formyl-4,5-dimethoxybenzoic acid, 490-63-1; acetanilide, 103-84-4.

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